Previous Page

Folic acid (FA, pteroyl-S-glutamic acid) *[75708-92-8]* **M 441.4, m >250°(dec), $[\alpha]_D^{25}$ +23° (c 0.5, 0.1N NaOH), pK_1 2.35 (protonation N10), pK_2 2.75 (protonation N1), pK_3 3.49 (α-CO_2H), pK_4 4.65 (γ-CO_2H), pK_5 8.80 (acidic N3).** If paper chromatography indicates impurities then recrystallise from hot H_2O or from dilute acid [Walker et al. *J Am Chem Soc* **70** 19 *1948*]. Impurities may be removed by repeated extraction with *n*-BuOH of a neutral aqueous solns of folic acid (by suspending in H_2O and adding N NaOH till the solid dissolves then adjusting the pH to ~7.0-7.5) followed by pptn with acid, filtration, and recrystn form hot H_2O. [Blakley *Biochem J* **65** 331 *1975*; Kalifa, Furrer, Bieri and Viscontini *Helv Chim Acta* **61** 2739 *1978*.] Chromatography on cellulose followed by filtration through charcoal has also been used to obtain pure acid. [Sakami and Knowles *Science* **129** 274 *1959*.] UV: λ_{max} 247 and 296nm (ε 12800 and 18700) in H_2O pH 1.0; 282 and 346nm (ε 27600 and 7200) in H_2O pH 7.0; 256, 284 and 366nm (ε 24600, 24500 and 8600) in H_2O pH 13 [Rabinowitz in *The Enzymes* (Boyer et al. Eds **2** 185 *1960*].

Follicle Stimulating Hormone (FSH, follitropin) *[9002-68-0]* **M_r ~36,000.** Purified by Sephadex G100 gel filtration followed by carboxymethyl-cellulose with NH_4OAc pH 5.5. The latter separates luteinising hormone from FSH. Solubility in H_2O is 0.5%. It has an isoelectric point of 4.5. A soln of 1mg in saline (100mL) can be kept at 60° for 0.5h. Activity is retained in a soln at pH 7-8 for 0.5h at 75°. The activity of a 50% aq EtOH soln is destroyed at 60° in 15 min. [Bloomfield et al. *Biochim Biophys Acta* **533** 371 *1978*; Hartree *Biochem J* **100** 754 *1966*; Pierce and Parsons *Ann Rev Biochem* **50** 465 *1981*.]

Fructose-1,6-diphosphate (trisodium salt) *[38099-82-0]* **M 406.1, pK_3^{25} 6.14, pK_4^{25} 6.93 (free acid).** For purification *via* the acid strychnine salt, see Neuberg, Lustig and Rothenberg [*Arch Biochem* **3** 33 *1943*]. The calcium salt can be partially purified by soln in ice-cold M HCl (1g per 10mL) and repptn by dropwise addition of 2M NaOH: the ppte and supernatant are heated on a boiling water bath for a short time, then filtered and the ppte is washed with hot water. The magnesium salt can be pptd from cold aqueous soln by adding four volumes of EtOH.

Fructose-6-phosphate *[643-13-0]* **M 260.1, $[\alpha]_D^{21}$ +2.5 (c 3, H_2O), pK^{25} 5.84.** Crystd as the barium salt from water by adding four volumes of EtOH. The barium can be removed by passage through the H^+ form of a cation exchange resin and the free acid collected by freeze-drying.

6-Furfurylaminopurine (Kinetin) *[525-79-1]* **M 215.2, m 266-267°, 269-271°, 270-272°, 272° (sealed capillary), pK_1 <1, pK_2 3.8, pK_3 10.** Platelets from EtOH and sublimes at 220°, but is best done at lower temperatures in a good vacuum. It has been extracted from neutral aqueous solns with Et_2O. [Miller et al *J Am Chem Soc* **78** 1375 *1956*; Bullock et al. *J Am Chem Soc* **78** 3693 *1956*.]

Fusaric acid (5-*n*-butylpyridine-2-carboxylic acid) *[536-69-6]* **M 179.2, m 96-98°, 98°, 98-100°, 101-103°, pK_1 5.7, pK_2 6.16 (80% aq methoxyethanol).** Dissolve in $CHCl_3$, dry (Na_2SO_4), filter, evaporate and recrystallise the residue from 50 parts of pet ether (b 40-60°) or EtOAc, then sublime *in vacuo*. The *copper salt* forms bluish violet crystals from H_2O and has **m** 258-259°. [Hardegger and Nikles *Helv Chim Acta* **39** 505 *1956*; Schreiber and Adam *Chem Ber* **93** 1848 *1960*; NMR and MS: Tschesche and Führer *Chem Ber* **111** 3500 *1978*.]

Fuschin (Magenta I, rosaniline HCl) *[632-99-5]* **M 337.9, m >200°(dec).** See rosaniline hydrochloride on p. 349 in Chapter 4.

D-Galactal

[21193-75-9] **M 146.2, m 100°, 100-102°, 104°, 103-106°, $[\alpha]_D^{20}$ -21.3° (c 1, MeOH).** Recryst from EtOAc, EtOH or EtOAc + MeOH. [Overend et al. *J Chem Soc* 675 *1950*; Wood and Fletcher *J Am Chem Soc* **79** 3234 *1957*; Distler and Jourdian *J Biol Chem* **248** 6772 *1973*.]

ß-Galatosidase (from bovine testes) *[9031-11-2]* **M_r 510,000, [EC 3.2.1.23].** Purified 600-fold by ammonium sulfate precipitation, acetone fractionation and affinity chromatography on agarose substituted with terminal thio-ß-galactopyranosyl residues. [Distlern and Jourdian *J Biol Chem* **248** 6772 *1973.*]

Gangcyclovir [9-{(1,3-dihydroxy-2-propoxy)methyl}guanine; 2-amino-1,9-{(2-hydroxy-1-hydroxymethyl)-ethoxymethyl}-6*H*-purin-6-one; Cytovene; Cymeva(e)n(e)] *[82410-32-0]* **M 255.2, m >290°(dec), >300°(dec), monohydrate m 248-249°(dec), $pK_{Est(1)}$~ -1.1, $pK_{Est(2)}$~ 4.1, $pK_{Est(3)}$~ 9.7.** Recryst from MeOH. Alternatively dissolve ~90g of reagent in 700mL of distilled H_2O, filter and cool (*ca* 94% recovery). UV: λ_{max} in MeOH 254nm (ε 12,880), 270sh nm (ε 9040), solubility in H_2O at 25° is 4.3mg/mL at pH 7.0. **ANTIVIRAL.** [Ogilvie et al. *Can J Chem* **60** 3005 *1982*; Ashton et al. *Biochem Biophys Res Commun* **108** 1716 *1982*; Martin et al. *J Med Chem* **26** 759 *1983.*]

Geranylgeranyl pyrophosphate *[6699-20-3 (NH_4 salt)]* **M 450.5, $pK_{Est(1)}$~<2, $pK_{Est(2)}$~<2, $pK_{Est(3)}$~3.95, $pK_{Est(4)}$~6.26.** Purified by counter-current distribution between two phases of a butanol/isopropyl ether/ammonia /water mixture (15:5:1:19) (v/v), or by chromatography on DEAE-cellulose (linear gradient of 0.02M KCl in 1mM Tris buffer, pH 8.9). Stored as a powder at 0°.

Geranyl pyrophosphate *[763-10-0 (NH_4 salt)]* **M 314.2, $pK_{Est(1)}$~<2, $pK_{Est(2)}$~<2, $pK_{Est(3)}$~3.95, $pK_{Est(4)}$~6.26.** Purified by paper chromatography on Whatman No 3 MM paper in a system of isopropyl alcohol/isobutyl alcohol/ammonia/water (40:20:1:39), R_F 0.77-0.82. Stored in the dark as the ammonium salt at 0°.

Gitoxigenin (3β,14,16β,21-tetrahydroxy-20(22)norcholenic acid lactone) *[545-26-6]* **M 390.5, m 223-226°, 234°, 239-240° (anhydrous by drying at 60°), $[\alpha]_D^{20}$ +30° (c 1, MeOH).** Recrystn from aqueous EtOH produces plates of the sesquihydrate which dehydrate on drying at 100° *in vacuo*. It has also been recrystd from Me_2CO-MeOH and from EtOAc the crystals contain 1 mol of EtOAc with $[\alpha]_D^{21}$ +24.8° (c 1, dioxane). It has UV has λ_{max} at 310, 485 and 520nm in 96% H_2SO_4. On heating with ethanolic HCl it yields *digitaligenin* with loss of H_2O. [Smith *J Chem Soc* 23 *1931.*]

Gliotoxin (3*R*-6*t*-hydroxy-3-hydroxymethyl-2-methyl-(5*at*)-2,3,6,10-tetrahydro-5*aH*-3,10*ac*-epidisulfido[1,2-*a*]-indol-1,4-dione) *[67-99-2]* **M 326.4, m 191-218°(dec), 220°(dec), 221°(dec), $[\alpha]_D^{20}$ -254° (c 0.6, $CHCl_3$), $[\alpha]_D^{25}$ -270° (c 1.7, pyridine).** Purified by recrystn from MeOH. Its solubility in $CHCl_3$ is 1%. The *dibenzoyl* derivative has **m** 202° (from $CHCl_3$-MeOH). [Glister and Williams *Nature* 153 651 *1944*; Elvidge and Spring *J Chem Soc* Suppl 135 *1949*; Johnson et al. *J Am Chem Soc* **65** 2005 *1943*; Bracken and Raistrick *Biochem J* **41** 569 *1947.*]

Glucose oxidase (from *Aspergillus niger*) *[9001-37-0]* **M_r 186,000, [EC 1.1.3.4].** Purified by dialysis against deionized water at 6° for 48hours, and by molecular exclusion chromatography with Sephadex G-25 at room temperature. [Holt and Cotton *J Am Chem Soc* **109** 1841 *1987.*]

Glucose-1-phosphate *[59-56-3]* **M 260.1, $[\alpha]_D^{25}$ +120° (c 3, H_2O), $[\alpha]_D^{20}$ +78° (c 4, H_2O of di-K salt), pK_1 1.11, pK_2 6.13 [pK^{25} 6.50]** . Two litres of 5% aq soln was brought to pH 3.5 with glacial acetic acid (+ 3g of charcoal, and filtered). An equal volume of EtOH was added, the pH was adjusted to 8.0 (glass electrode) and the soln was stored at 3° overnight. The ppte was filtered off, dissolved in 1.2L of distd water, filtered and an equal volume of EtOH was added. After standing at 0° overnight, the crystals were collected at the centrifuge, and washed with 95% EtOH, then absolute EtOH, ethanol/diethyl ether (1:1), and diethyl ether. [Sutherland and Wosilait, *J Biol Chem* **218** 459 *1956.*] Its barium salt can be crystd from water and EtOH. Heavy metal impurities can be removed by passage of an aqueous soln (*ca* 1%) through an Amberlite IR-120 column (in the appropriate H^+, Na^+ or K^+ forms). *Di-K salt* cryst as $2H_2O$ from EtOH.

Glucose-6-phosphate *[acid 156-73-5; Ba salt 58823-95-3; Na salt 54010-71-8]* **M 260.1, m 205-207°(dec) mono Na salt, $[\alpha]_{546}^{20}$ +41° (c 5, H_2O), pK_1 1.65, pK_2 6.11, pK_3^{25} 11.71 [-C_1(OH)O⁻].** Can be freed from metal impurities as described for glucose-1-phosphate. Sol of Na Salt is 5% in H_2O at 20°. Its barium salt can be purified by solution in dilute HCl and pptn by neutralising the soln. The ppte is washed with small volumes of cold water and dried in air.

Glucose-6-phosphate dehydrogenase *[9001-40-5]* **M_r 128,000 (from Baker's yeast), 63,300 (from rat mammary gland) [EC 1.1.1.49].** The enzyme is useful for measuring pyridine nucleotides in enzyme recycling. The enzyme from Baker's yeast has been purified by $(NH_4)_2SO_4$ fractionation, Me_2CO pptn, a second $(NH_4)_2SO_4$ fractionation, concentration by DEAE-SF chromatography, a third $(NH_4)_2SO_4$ fractionation and recrystn. Crystn is induced by addition of its coenzyme NADP, which in its presence causes rapid separation of crystals at $(NH_4)_2SO_4$ concentration much below than required to ppte the amorphous enzyme. To recryst, the crystals are dissolved in 0.01M NADP (pH 7.3) with $(NH_4)_2SO_4$ at 0.55 saturation and the crystals appear within 10 to 60 min. After standing for 2-3 days (at 4°) the $(NH_4)_2SO_4$ is increased to 0.60 of saturation and more than 80% of the activity in the original crystals is recovered in the fresh crystals. [Noltmann et al. *J Biol Chem* **236** 1255 *1961*]. Large amounts can be obtained from rat livers. The livers are extracted with 0.025M phosphate buffer (pH 7.5), and ppted with 3M $(NH_4)_2SO_4$ (70% of activity). The ppte is dissolved in 3volumes of 0.025M phosphate (pH 7.5), dialysed against this buffer + 0.2mM EDTA at 4° for 5h, then diluted to 1% protein and the nucleic acids ppted by addition of 0.4volumes of 1% protamine sulfate. $(NH_4)_2SO_4$ is added to a concentration of 2M (pH adjusted to 7.0 with NH_3), the ppte is discarded and the supernatant is adjusted to 2.8M $(NH_4)_2SO_4$, dialysed, protein adjusted to 1% and treated with $Ca_3(PO_4)_2$ gel. The gel is added in three steps (1.5mL of 0.4% gel/mL per step) and the gel is removed by centrifugation after each addn. The third gel adsorbed 50% of the activity. The gel is eluted with 0.2M phosphate (pH 7.4, 40mL/g of gel; 60% recovery). The extract is ppted in 3volumes with $(NH_4)_2SO_4$ (adjusted to 4M) to give enzyme with an activity of 30μmoles/mg of protein x hour. [Lowry et al. *J Biol Chem* **236** 2746 *1961*.] Km values for the yeast enzyme are 20μM for G-6P and 2μM for NADP (Tris pH 8.0, 10^{-2} M $MgCl_2$, 38°) [Noltmann and Kuby *The Enzymes* **VII** 223 *1963*].

L-Glutathione (reduced form, γ-L-glutamyl-L-cysteinyl-glycine) *[70-18-8]* **M 307.3, m 188-190°(dec), 195°(dec), $[\alpha]_D^{20}$-20.1° (c 1, H_2O), pK_1^{25} 2.12 (CO_2H), pK_2^{25} 3.59 (CO_2H), pK_3^{25} 8.75 (NH_2), pK_4^{25} 9.65 (10.0, SH).** Crystd from 50% aq EtOH, dry in a vac and store below 5°. Alternatively recrystd from aqueous EtOH under N_2, and stored dry in a sealed container below 4°. It is soluble in H_2O. [Weygand and Geiger *Chem Ber* **90** 634 *1957*; Martin and Edsall *Bull Soc Chim Fr* **40** 1763 *1958*; *Biochem Prep* **2** 87 *1952*.]

L-Glutathione (oxidised) *[27025-41-8]* **M 612.6, m 175-195°, 195°, $[\alpha]_D^{20}$-98° (c 2, H_2O), pK_1 3.15, pK_2 4.03, pK_3 8.75.** Purified by recrystn from 50% aq EtOH. Its solubility in H_2O is 5%. Store at 4°. [Li et al. *J Am Chem Soc* **76** 225 *1954*; Berse et al. *Can J Chem* **37** 1733 *1959*.]

Glutathione S-transferase (human liver) *[50812-37-8]* **M_r 25,000, [EC 2.5.1.18].** Purified by affinity chromatography using a column prepared by coupling glutathione to epoxy-saturated Sepharose. After washing contaminating proteins the pure transferase is eluted with buffer containing reduced glutathione. The solution is then concentrated by ultrafiltration, dialysed against phosphate buffer at pH ~7 and stored in the presence of dithiothreitol (2mM) in aliquots at <-20°. [Simons and Vander Jag *Anal Biochem* **52** 334 *1977*.]

Glyceraldehyde-3-phosphate dehydrogenase *[9001-50-7]* **M_r 144,000, [EC 1.2.1.12].** Purified from rabbit muscle by extraction with 0.03N KOH and ppted with $(NH_4)_2SO_4$ (0.52 of saturation). The clear supernatant was adjusted to pH 7.5 and NH_3 was added dropwise to pH 8.2-8.4. Crystals appear sometimes even without seeding. The crystals are dissolved in H_2O, filtered to remove suspended material and 2 volumes of saturated $(NH_4)_2SO_4$ at pH 8.2-8.4 is added. After 1hour the crystals appear. Recrystallise in the same way. [Cori et al. *J Biol Chem* **173** 605 *1948*; Furfine and Velick *J Biol Chem* **240** 844 *1965, The Enzymes* **7** 243 *1963*; Lui and Huskey *Biochemistry* **31** 6998 *1992*.] The Km values are: NADH (3.3μM) and 1,3-diphosphoglycerate ($8x10^{-7}$M) in pH 7.4 imidazole buffer at 26°, NAD (13μM), glyceraldehyde-3-P (90μM), P_i ($2.9x10^{-4}$M), and arsenate (69μM) in 8.6 M $NaHCO_3$ buffer at 26°. [Orsi and Cleland *Biochemistry* **11** 102 *1972*.]

Glycerol kinase (from *Candida mycoderma*, *E coli*, rat or pigeon liver glycerokinase) *[9030-66-4]* **M_r 251,000, [EC 2.7.1.30].** Commercial enzyme has been dialysed against 2mM Hepes, 5mM dithiothreitol and 0.3mM EDTA, followed by several changes of 20mM Hepes and 5mM dithiothreitol prior to storage under N_2 at -20°. [Knight and Cleland *Biochemistry* **28** 5728 *1989*.] The enzyme from pigeon liver was purified by acid-pptn (acetate buffer at pH 5.1), $(NH_4)_2SO_4$ fractionation, heat treatment (60°/ 1 h),

calcium phosphate gel filtration, a second $(NH_4)_2SO_4$ fractionation, dialysis, elution of inert proteins and crystn. This was done by repeatedly extracting the ppte from the last step with 0.05M sodium pyrophosphate (pH 7.5) containing 1mM EDTA and 0.2M $(NH_4)_2SO_4$ was added. Careful addition of solid $(NH_4)_2SO_4$ to this soln lead to crystn of the enzyme. Recrystn was repeated. The enzyme is activated by Mg^{2+} and Mn^{2+} ions and is most stable in solns in the pH 4.5-5.5 range. The stability is greatly increased in the presence of glycerol. It has Km for glycerol of 60μM and for ATP 9μM in glycine buffer pH 9.8 and 25°. [Kennedy *Methods Enzymol* **5** 476 *1962*.]

L-Glycerol-3-phosphate dehydrogenase (GDH, from rabbit muscle) *[9075-65-4]* **M_r 78,000 [EC 1.1.1.8].** Recrystd by adding $(NH_4)_2SO_4$ till 0.45 saturation at pH 5.5 at 4° and the small amount of ppte is removed then satd $(NH_4)_2SO_4$ is added dropwise from time to time over several days in the cold room. The crystals are collected and recrystd until they have maximum activity. The enzyme is stable in half saturated $(NH_4)_2SO_4$ for several weeks at 4°. The equilibrium [dihydroxyacetone][NADH][H^+]/[G-3-P][NAD] is 1.0 x 10^{-12}M in Tris buffer at 25°. It uses NAD ten times more efficiently than NADP. The Km for G-3-P is 1.1 x 10^{-4}M, for NAD it is 3.8 x 10^{-4}M and for dihydroxyacetone it is 4.6 x 10^{-4}M in phosphate buffer pH 7.0 and at 23.3°. Dihydroxyacetone phosphate and fructose-1,6-diphosphate are inhibitors. [Branowski *J Biol Chem* **180** 515 *1949*, *The Enzymes* **7** 85 *1963*; Young and Pace *Arch Biochem Biophys* **75** 125 *1958*; Walsh and Sallach *Biochemistry* **4** 1076 *1965*.]

L-α-Glycerol phosphocholine (Cadmium Chloride)$_x$ complex *[64681-08-9]* **M 257.2 + (183.3)$_x$, pK_{Est} ~ 5.5.** Glycerol phosphocholine is purified *via* the $CdCl_2$ complex which is purified by four recrystns from 99% EtOH by standing at 0° for 1h. The white ppte is collected, washed with EtOH, Et_2O and dried in a vacuum. The amorphous Cd complex can be converted to the crystalline form [$C_8H_{20}O_6NP.CdCl_2.3H_2O$] by dissolving 34.4g in H_2O (410mL) and 99% EtOH (1650mL total) added slowly with stirring and allowing the clear soln to stand at 25° for 12hours, then at 5° for 12h. The crystallised complex is filtered off, washed with cold 80% EtOH and dried in air. Glycerol phosphocholine can be recovered from the complex by dissolving in H_2O (2% soln), passing through an ion-exchange column (4.9 x 100cm, of 1vol IRC-50 and 2vol of IR-45). The effluent is concentrated to a thick syrup at 45°. It is dried further at 50°/P_2O_5/48h. The vitreous product (~8.25g) is dissolved in 99% EtOH (50mL) and the clear soln is cooled at 5°, whereby crystals appear, and then at -15° for 16h. The crystals are filtered off, washed with 99% EtOH, and Et_2O then dried at 50° in a vacuum over P_2O_5. It can be recrystd from 99.5% EtOH, long prisms) which are *hygroscopic* and must be handled in a H_2O-free atmosphere [Tattrie and McArthur *Biochem Prep* **6** 16 *1958*; Baer and Kates *J Am Chem Soc* **70** 1394 *1948* ; *Acta Cryst* **21** 79, 87 *1966*].

Glycine anhydride (2,5-diketopiperazine) *[106-57-0]* **M 114.1, m 309-310°, 311-312°(dec), ~315°(dec), pK_1 -4.45, pK_2 -2.16 (pK_2 -1.94 in AcOH).** Recrystd from H_2O (plates) and can be sublimed (slowly) at 260° or at 140-170°/0.5mm. The *dihydrochloride* has **m** 129-130°, is prepd by dissolving in conc HCl and on adding EtOH to crystallisation point; dried in a vac. The *bis-1-naphthylurethane* has **m** 232°(dec), and the *diperchlorate* has **m** 117° (*hygroscopic*). [MS: Johnstone *J Chem Soc Perkin Trans 1* 1297 *1975*; NMR: Blaha and Samek *Collect Czech Chem Commun* **32** 3780 *1967*; Sauborn *J Phys Chem* **36** 179 *1932*; Corey *J Am Chem Soc* **60** 1599 *1938*.]

Glycocyamine (*N*-guanylglycine) *[352-97-6]* **M 117.1, m 280-284°(dec), >300°, pK^{25} 2.86 (NH_3^+).** Recrystd from 15 parts of hot H_2O, or by dissolving in slightly more than the calculated amount of 2N HCl and ppting by adding an equivalent of 2N NaOH, filtering washing with cold H_2O and drying first *in vacuo* then at 60° *in vacuo*. The *hydrochloride* has **m** 200°(dec) after recrystn from aqueous HCl as plates. The *picrate* forms needles from hot H_2O and has **m** 210°(dec). [Brand and Brand *Org Synth* Coll Vol III 440 *1955*; Failey and Brand *J Biol Chem* **102** 768 *1933*; King *J Chem Soc* 2375 *1930*.]

Glycodeoxycholic acid monohydrate (*N*-[3α-12α-dihydroxy-5β-cholan-24-oyl]glycine) *[360-65-6]* **M 467.6, m 186-177°(dec), 187-188°, $[\alpha]_D^{23}$ +45.9° (c 1, EtOH), pK_{Est} ~ 4.4.** Recrystallises from H_2O or aqueous EtOH with 1 mol of H_2O and dried at 100° *in vacuo*. Solubility in EtOH is 5%. [UV: Lindstedt and Sjövall *Acta Chem Scand* **11** 421 *1957*.] The *Na salt* is recrystd from EtOH/Et_2O, **m** 245-250°, $[\alpha]_D^{23}$ +41.2° (c 1, H_2O) [Wieland *Hoppe Seyler's Z Physiol Chem* **106** 181 *1919*; Cortese *J Am Chem Soc* **59** 2532 *1937*].

D(+)-Glycogen *[9005-79-2]* **M 25,000-100,000, m 270-280°(dec), $[\alpha]_{546}$ +216° (c 5, H_2O).** A 5% aqueous soln (charcoal) was filtered and an equal volume of EtOH was added. After standing overnight at 3° the ppte was collected by centrifugation and washed with absolute EtOH, then EtOH/diethyl ether (1:1), and diethyl ether. [Sutherland and Wosilait *J Biol Chem* **218** 459 *1956.*]

Glycogen synthase (from bovine heart) *[9014-56-6]* **M_r 60,000, [EC 2.4.1.11].** Purified by pptn of the enzyme in the presence of added glycogen by polyethylene glycol, chromatography on DEAE-Sephacel and high speed centrifugation through a sucrose-containing buffer. [Dickey-Dunkirk and Kollilea *Anal Biochem* **146** 199 *1985.*]

Gramicidin A (a pentadecapeptide from *Bacillus brevis*) *[11029-61-1]* **m ~229-230°(dec).** Purified by countercurrent distribution from $^*C_6H_6$-$CHCl_3$, MeOH-H_2O (15:15:23:7) with 5000 tubes. Fractions were examined by UV (280nm) of small aliquots. Separation from Gramicidin C and other material occurred after 999 transfers. [Gross and Witkop *Biochemistry* **4** 2495 *1965*; Bauer et al. *Biochemistry* **11** 3266 *1972.*] Purified finally by recrystn from EtOH-H_2O and dried at 100°/10^{-2} mm over KOH and forms platelets **m** 229-230°. Almost insoluble in H_2O (0.6%) but soluble in lower alcohols, dry Me_2CO, dioxane, acetic acid and pyridine. The commercial material is more difficult to crystallise than the synthetic compound. [Sarges and Witkop *J Am Chem Soc* **86** 1861, **87** 2011, 2020 *1965.*] It has characteristic $[\alpha]_D^{20}$ +27.3° (c 1.3, MeOH) and UV λ_{max} 282nm (ε 22,100). The *N-carbamoyldeformyl gramicidine A* pptes from EtOAc-pet ether (b 40-60°).

Gramicidin C (gramicidin S, a pentadecapeptide from *Bacillus brevis*) *[9062-61-7].* Same as Gramicidin A since they are isolated together and separated. [Sarges and Witkop *Biochemistry* **4** 2491 *1965*; Hunter and Schwartz "Gramicidins" in *Antibotics I* (Gotlieb and Shaw Eds) Springer-Verlag, NY, p.642 *1967;* as well as references above for Gramicidin A.]

Gramicidin S 2HCl (from *Bacillus brevis* Nagano) *[15207-30-4]* **M 1214.4, m 277-278°(dec), $[\alpha]_D^{24}$ -289° (c 0.4, 70% aq EtOH).** Crysts in prisms from EtOH + aq HCl.

Gramicidin S *[113-73-5]* **M 1141.4, m 268-270°, $[\alpha]_D^{25}$ -290° (c 0.5, EtOH + 30mM aq HCl {7:3}].** Crystd from EtOH. *Di-HCl [15207-30-4]* cryst from EtOH (+ few drops of HCl) has **m** 277-278°.

***N*-Guanyltyramine hydrochloride** *[60-20-8]* **M 215.7, m 218°, pK_1 10.2 (phenolic OH), pK_2 12.4 (guanidino N).** Purified on a phosphocellulose column and eluted with a gradient of aqueous NH_3 (0-10%). The second major peak has the characteristic tryptamine spectrum and is collected, lyphilised to give white crystals of the *dihydrate* which dehydrates at 100°. It has UV λ_{max} at 274.5nm (ε 1310) in 0.1N NaOH and 274.5nm (ε 1330) at pH 7.0. Excitation λ_{max} is at 280nm and emission λ_{max} is at 330nm. [Mekalanos et al. *J Biol Chem* **254** 5849 *1979.*]

Haemoglobin A (from normal human blood) *[9008-02-0]* M_r ~64,500, amorphous.

Purified from blood using CM-32 cellulose column chromatography. [Matsukawa et al. *J Am Chem Soc* **107** 1108 *1985.*] For the purification of the α and β chains see Hill et al. *Biochem Prep* **10** 55 *1963.*

Harmaline (7-methoxy-1-methyl-4,9-dihydro-3*H*-β-carboline, 4,9-dihydro-7-methoxy-1-methyl-3*H*-pyrido[3,4-*b*]indole) *[304-21-2]* **M 214.3, m 229-230°, 229-231°, 235-237° (after distn at 120-140°/10^{-3}), pK_1 4.2.** Recrystd from MeOH and sublimed at high vacuum. It has UV in MeOH has λ_{max} 218, 260 and 376nm (log ε 4.27, 3.90 and 4.02 respectively); IR (Nujol) ν 1620, 1600, 1570 and 1535cm^{-1} and in $CHCl_3$ ν 1470 and 1629cm^{-1}. [Spenser *Can J Chem* **37** 1851 *1959*; Marion et al. *J Am Chem Soc* **73** 305 *1951*; UV Prukner and Witkop *Justus Liebigs Ann Chem* **554** 127 *1942.*] The *hydrochloride dihydrate* has **m** 234-236°(dec), the *picrate* has **m** 228-229° (sinters at 215°) from aqueous EtOH, and the *N-acetate* forms needles **m** 204-205°.

Hematin (ferrihaeme hydroxide) *[15489-90-4]* **M 633.5, m 200°(dec), pK_{Est} ~ 4.** Crystd from pyridine. Dried at 40° *in vacuo.*

Hematoporphyrin (3,3'-[7,12-bis-(1-hydroxyethyl)-3,8,13,17-tetramethyl-porphyrin-2,18-diyl]-dipropionic acid) *[14459-29-1]* **M 598.7, pK_{Est} ~4.8.** Purified by dissolving in EtOH and adding H_2O or Et_2O to give deep red crystals. Also recrystd from MeOH. UV has λ_{max} at 615.5, 565, 534.4 and 499.5nm in 0.1 N NaOH, and 597, 619, 634,653, 683 and 701nm in 2 N HCl. [Falk *Porphyrins and Metalloporphyrins* Elsevier, NY, p 175 *1964.*] It is used in the affinity chromatographic purification of Heme proteins [Olsen *Methods Enzymol* 123 324 *1986.*] The *O-methyl-dimethyl ester* has **m** 203-206° (from $CHCl_3$-MeOH) and the *O,O'-dimethyl-dimethyl ester* has **m** 145° (from $CHCl_3$-MeOH). [Paul *Acta Chem Scand* **5** 389 *1951.*]

Hematoporphyrin dimethyl ester *[33070-12-1]* **M 626.7, m 212°.** Crystd from $CHCl_3$/MeOH.

Hematoxylin (±-11*bc*-7,11*b*-dihydroindeno[2,1-*c*]-chromen-3,4-6*ar*-9,10-pentaol) *[517-28-2]* **M 302.3, m 200°(dec), 210-212°(dec).** Recrystd from H_2O (as trihydrate) in white-yellow crystals which become red on exposure to light and then melt at 100-120°. It has been recrystd from Me_2CO-$^*C_6H_6$. Crystd also from dil aqueous $NaHSO_3$ until colourless. Soluble in alkali, borax and glycerol. Store in the dark below 0°. [Morsingh and Robinson *Tetrahedron* **26** 182 *1970*; Dann and Hofmann *Chem Ber* **98** 1498 *1955.*]

Hemin (ferriproptoporphyrin IX chloride) *[16009-13-5]* **M 652.0, m sinters at 240°, pK_{Est} ~4.8.** It is purified by recrystn from AcOH. Also heme (5g) is shaken in pyridine (25mL) till it dissolves, then $CHCl_3$ (40mL) is added, the container is stoppered and shaken for 5min (releasing the stopper occasionally). The soln is filtered under slight suction, and the flask and filter washed with a little $CHCl_3$ (15mL). During this period, AcOH (300mL) is heated to boiling and saturated aqueous NaCl (5mL) and conc HCl (4mL) are added. The $CHCl_3$ filtrate is poured in a steady stream, with stirring, into the hot AcOH mixture and set aside for 12hours. The crystals are filtered off, washed with 50% aqueous AcOH (50mL), H_2O (100mL), EtOH (25mL), Et_2O and dried in air. [Fischer *Org Synth* Coll Vol III 442 *1955.*]

Heparin (from pig intestinal mucosa) *[9005-49-6]* **M_r ~3,000, amorphous, $[\alpha]_D^{20}$ ~+55° (H_2O).** Most likely contaminants are mucopolysaccharides including heparin sulfate and dermatan sulfate. Purified by pptn with cetylpyridinium chloride from saturated solutions of high ionic strength. [Cifonelli and Roden *Biochem Prep* **12** 12 *1968.*]

Heparin (sodium salt) *[9041-08-1]* **M_r ~ 3000 (low Mol Wt, Bovine), amorphous, $[\alpha]_D^{20}$ +47° (c 1.5, H_2O).** Dissolved in 0.1M NaCl (1g/100mL) and ppted by addition of EtOH (150mL).

Histones (from S4A mouse lymphoma). Purification used a macroprocess column, heptafluorobutyric acid as solubilising and ion-pairing agent and an acetonitrile gradient. [McCroskey et al. *Anal Biochem* **163** 427 *1987.*]

Hyaluronidase *[9001-54-1, 37326-33-3]* **M_r 43,000 (bovine testes), 89,000 (bacterial), [EC 3.2.1.35].** Purified by chromatography on DEAE-cellulose prior to use. [Distler and Jourdain *J Biol Chem* **248** 6772 *1973.*]

Hydrocortisone (11β,17α,21-trihydroxy-pregn-4-ene-3,20-dione) *[50-23-7]* **M 362.5, m 212-213°, 214-217°, 218-221°, 220-222°, $[\alpha]_D^{22}$+167° (c 1, EtOH).** Recrystd from EtOH or isoPrOH. It is bitter tasting and has UV λ_{max} at 242 nm (log ε 4.20). Its solubility at 25° is: H_2O (0.28%), EtOH (1.5%), MeOH (0.62%), Me_2CO (0.93%), $CHCl_3$ (0.16%), propylene glycol (1.3%) and Et_2O (0.35%). It gives an intense green colour with conc H_2SO_4. [Wendler et al. *J Am Chem Soc* **72** 5793 *1950.*]

Hydrocortisone acetate (21-acetoxy-11β,17α-trihydroxy-pregn-4-ene-3,20-dione) *[50-03-3]* **M 404.5, m 218-221.5°, 221-223°, 222-225°, $[\alpha]_D^{25}$+166° (c 0.4, dioxane), +150.7° (c 0.5, Me_2CO).** Recrystd from Me_2CO-Et_2O or aqueous Me_2CO as somewhat *hygroscopic* monoclinic crystals. UV has λ_{max} 242 nm ($A_{1cm}^{1\%}$ 390) in MeOH. Its solubility at 25° is: H_2O (0.001%), EtOH (0.45%), MeOH (0.04%), Me_2CO (1.1%), $CHCl_3$ (0.5%), Et_2O (0.15%) and is very soluble in Me_2NCHO. [Wendler et al. *J Am Chem Soc* **74** 3630 *1952*; Antonucci et al. *J Org Chem* **18** 7081 *1953.*]

(+)-Hydroquinidine anhydrous (9*S*-6'-methoxy-10,11-dihydrocinchonan-9-ol) *[1435-55-8]* **M 326.4, m 168-169°, 169°, 169-170°, 171-172°, $[\alpha]_D^{20}$ +231° (c 2, EtOH), +299° (c 0.82, 0.1N H_2SO_4), pK_{Est} ~ 8.8.** Forms needles from EtOH and plates from Et_2O. Slightly soluble in Et_2O and H_2O but readily soluble in hot EtOH. [Heidelberger and Jacobs *J Am Chem Soc* **41** 826 *1919*; King *J Chem Soc* 523 *1946*.] The *hydrochloride* has **m** 273-274°, $[\alpha]_D^{26}$ +184° (c 1.3, MeOH) and is very soluble in MeOH and $CHCl_3$,, but less soluble in H_2O, EtOH and less soluble in dry Me_2CO. [Kyker and Lewis *J Biol Chem* **157** 707 *1945*; Emde *Helv Chim Acta* **15** 557 *1932*.]

Hydroquinine *[522-66-7]* **M 326.4, m 168-171°, 171.5°, $[\alpha]_D^{16}$ +143° (c 1.087, EtOH), pK^{15} 8.87.** Recrystd from EtOH. [Rabe and Schultz *Chem Ber* **66** 120 *1933*.]

19-Hydroxy-4-androsten-3,17-dione *[510-64-5]* **M 302.4, m 167-169°, 168-170°, 169-170°, 172-173°, $[\alpha]_D^{20}$ +190° (c 1, $CHCl_3$).** Recrystd from Me_2CO-hexane or Et_2O-hexane. It has UV λ_{max} at 242nm in EtOH or MeOH. The *19-acetoxy* derivative has $[\alpha]_D^{26}$ +185° ($CHCl_3$) and λ_{max} 237.5nm in EtOH. [Ehrenstein and Dünnenberger *J Org Chem* **21** 774 *1956*.]

3-Hydroxy butyrate dehydrogenase (from *Rhodopseudomonas spheroides*) *[9028-38-0]* **M_r ~85,000, [EC 1.1.1.30], amorphous.** Purified by two sequential chromatography steps on two triazine dye-Sepharose matrices. [Scavan et al. *Biochem J* **203** 699 *1982*.]

25-Hydroxycholesterol (cholest-5-en-3β,25-diol) *[2140-46-7]* **M 402.7, m 177-179°, 178-180°, 181.5-182.5°, $[\alpha]_D^{25}$ -39° (c 1.05, $CHCl_3$).** Forms colourless needles from MeOH. [Schwartz *Tetrahedron Lett* **22** 4655 *1981*.] The *3β–acetoxy derivative* has **m** 142-142.8° (from Me_2CO), $[\alpha]_D^{25}$ -40.4° (c 2, $CHCl_3$). The *3β,25-diacetyl derivative* has **m** 119-120.5° (from MeOH), $[\alpha]_D^{25}$ -35.5° ($CHCl_3$). [Dauben and Bradlow *J Am Chem Soc* **72** 4248 *1950*; Ryer et al. *J Am Chem Soc* **72** 4247 *1950*.]

18-Hydroxy-11-deoxycorticosterone (18,21-dihydroxypregn-4-en-3,20-dione tautomeric with 18,20-epoxy-20,21-dihydroxypregn-4-en-3-one) *[379-68-0]* **M 346.5, m 168-170°, 171-173°, 191-195°, 200-205°, $[\alpha]_D^{20}$ +151° (c 1, $CHCl_3$).** Recrystn from Et_2O-Me_2CO gave crystals **m** 200-205°, when recrystd from M_2CO it had **m** 191-195°. It has UV λ_{max} at 240nm. The *21-O-acetoxy-18-hydroxy derivative* has **m** 158-159° (from Et_2O-*C_6H_6) and the *21-O-acetoxy-18,20-epoxy derivative* has **m** 149-154° (from Et_2O). [Kahnt et al. *Helv Chim Acta* **38** 1237 *1955*; Pappo *J Am Chem Soc* **81** 1010 *1959*.]

***R*-(-)-2-Hydroxy-3,3-dimethyl-γ-butyrolactone (3-hydroxy-4,4-dimethyl-4,5-dihydrofuran-2-one, D-pantolactone)** *[599-04-2]* **M 130.1, m 89-91°, 90.5-91.5°, 91°, 92-93°, b 120-122°/15mm, $[\alpha]_{D*}^{20}$ -28° (c 5, MeOH), $[\alpha]_D^{20}$ -51° (c 3, H_2O).** Recrystallise from Et_2O-pet ether, diisopropyl ether or *C_6H_6-pet ether and sublime at 25°/0.0001mm. It hydrolyses readily to the hydroxy-acid and racemises when heated above 145°. The *Brucine salt* has **m** 211-212° (from EtOH). [Kuhn and Wieland *Chem Ber* **73** 1134 *1940*; and Stiller et al. *J Am Chem Soc* **62** 1779 *1940*; Bental and Tishler *J Am Chem Soc* **68** 1463 *1946*.]

(±)-Ibotenic acid monohydrate

(α-[3-hdyroxy-5-isoxazolyl]-glycine, α-amino-3-hydroxy-5-isoxazoleacetic acid) *[2552-55-8]* **M 176.1, m 144-146° (monohydrate), 151-152° (anhydrous), 148-151°, pK_1 2, pK_2 5.1, pK_3 8.2.** It has been converted to the ammonium salt (**m** 121-123° dec) dissolved in H_2O and passed through an Amberlite IR 120 resin (H^+ form) and eluted with H_2O. The acidic fractions were collected, evaporated to dryness and the residue recrystd from H_2O as the monohydrate (**m** 144-146°). The anhydrous acid is obtained by making a slurry with MeOH, decanting and evaporating to dryness and repeating the process twice more to give the anhydrous acid (**m** 151-152°). Recrystn from H_2O gives the monohydrate. [Nakamura *Chem Pharm Bull Jpn* **19** 46 *1971*.] The *ethyl ester* forms needles when crystd from a small volume of Et_2O and has **m** 78-79° and IR ($CHCl_3$) with ν 3500–2300 (OH), 1742 (ester CO), 1628, 1528cm^{-1}, and UV with λ_{max} (EtOH) at 206nm (ε 7080). The *hydrazide* has **m** 174-175° (from MeOH) with IR (KBr) 1656 (C=O)cm^{-1}.

2-Iminothiolane hydrochloride (2-iminotetrahydrothiophene) *[4781-83-3]* **M 137.6, m 187-192°, 190-195°, 193-194°, 202-203°, pK <2 (free base).** Recryst from MeOH-Et_2O (**m** 187-192°) but after sublimation at ~180°/0.2mm the melting point rose to 202-203°. It has NMR with δ 2.27 (2H, t), 3.25 (2H, t) and 3.52 (2H, t) in $(CD_3)_2SO$. [King et al. *Biochemistry* **17** 1499 *1978*.] The *free base* is purifed by vacuum distn (**b** 71-72°/6mm) with IR (film) with ν 1700 (C=N)cm^{-1} and NMR ($CDCl_3$) with δ at 3.58 (2H, t) and 2.10-2.8 (4H, m). The *free base* is stable on storage but slowly hydrolyses in aqueous solns with half lives at 25° of 390h at pH 9.1, 210h at pH 10 and 18 h at pH 11. [Alagon and King *Biochemistry* **19** 4343 *1980*.]

***trans*-Indol-3-ylacrylic acid** *[1204-06-4]* **M 187.2, m 190-195°(dec), 195°(dec), 196°(dec), 195-196°(dec), pK_{Est} ~ 4.2.** Recrystd from AcOH, H_2O or EtOAc-cyclohexane. UV in MeOH has λ_{max} at 225, 274 and 325nm. [Shaw et al. *J Org Chem* **23** 1171 *1958*; constitution: Rappe *Acta Chem Scand* **18** 818 *1964*; Moffatt *J Chem Soc* 1442 *1957*; Kimming et al. *Hoppe Seyler's Z Physiol Chem* **371** 234 *1958*.]

3-Indolylbutyric acid *[133-32-4]* **M 203.2, m 120-123°, 123-125°, 124°, pK 4.84.** Recrystd from H_2O. It is soluble in EtOH, Et_2O and Me_2CO but insoluble in $CHCl_3$. [Bowman and Islip *Chem Ind London* 154 *1971*; Jackson and Manske *J Am Chem Soc* **52** 5029 *1930*; Albaum and Kaiser *Am J Bot* **24** 420 *1937*.] UV has λ_{max} 278 and 320nm in isoPrOH [Elvidge *Quart J Pharm Pharmacol* **13** 219 *1940*]. The *methyl ester* has **m** 73-74° (from $^*C_6H_6$-pet ether) and **b** 230°/6mm [Bullock and Hand *J Am Chem Soc* **78** 5854 *1951*]. Also recrystd from EtOH/water [James and Ware *J Phys Chem* **89** 5450 *1985*].

3-Indolylpyruvic acid *[392-12-1]* **M 203.2, m~210°(dec), 208-210°(dec), 219°(dec), pK_{Est} ~ 2.4.** Recrystd from Me_2CO-$^*C_6H_6$, EtOAc-$CHCl_3$, Me_2CO-AcOH (crystals with 1 molecule of AcOH) and dioxane-$^*C_6H_6$ (with 0.5 molecule of dioxane) [Shaw et al. *J Org Chem* **23** 1171 *1958*; Kaper and Veldstra *Biochim Biophys Acta* **30** 401 *1958*]. The *ethyl ester* has **m** 133° (from Et_2O) and its *2,4-dinitrophenylhydrazone* has **m** 255° (from Me_2CO). [Baker *J Chem Soc* 461 *1946*.]

***myo*-Inositol (cyclohexane[1*r*,2*c*,3*c*,4*t*,5*c*,6*t*]-hexol)** *[87-89-8]* **M 180.2, m 218° (dihydrate), 225-227°, 226-230°.** Recrystd from aq 50% ethanol or H_2O forming a dihydrate, or anhydrous crystals from AcOH. The dihydrate is efflorescent and becomes anhydrous when heated at 100°. The anhydrous crystals are not hygroscopic. Solubility in H_2O at 25° is 14%, at 60° it is 28%, slightly soluble in EtOH but insoluble in Et_2O. [Ballou and Anderson *J Am Chem Soc* **75** 748 *1953*; Anderson and Wallis *J Am Chem Soc* **70** 2931 *1948*.]

Interferons [αIFN, βIFN and γIFN]. Interferons are a family of glycosylated proteins and are cytokines which are produced a few hours after cells have been infected with a virus. Interferons protect cells from viral infections and have antiviral activities at very low concentrations (~3 x 10^{-4} M, less than 50 molecules are apparently sufficient to protect a single cell). Double stranded RNA are very efficient inducers of IFNs. There are three main types of IFNs. The αIFNs are synthesised in lymphocytes and the βIFNs are formed in infected fibroblasts. The α and β families are fairly similar consisting of *ca* 166 to 169 amino acids. Although γIFNs are also small glycosylated proteins (*ca* 146 amino acids), they are different because they are not synthesised after viral infections but are produced by lymphocytes when stimulated by **mitogens** (agents that induced cell division).
Several of these IFNs of mouse and human lymphocytes and fibroblasts are available commercially and have been best prepared in quantity by recombinant DNA procedures because they are produced in very small amounts by the cells. The commercial materials do not generally require further purification for their intended purposes. [Pestkas, Interferons and Interferon standards and general abbreviations, *Methods Enzymol*, Wiley & Sons, **119** *1986*, ISBN 012182019X; Lengyel, Biochemistry of interferons and their actions, *Ann Rev Biochem* **51** 251-282 *1982*; De Maeyer and De Maeyer-Guignard, Interferons in *The Cytokine Handbook*, 3rd Edn, Thomson et al. Eds, pp. 491-516 *1998* Academic Press, San Diego, ISBN 0126896623.]

Interleukin (from human source). Purified using lyophilisation and desalting on a Bio-Rad P-6DC desalting gel, then two steps of HPLC, first with hydroxylapatite, followed by a TSK-125 size exclusion column. [Kock and Luger *J Chromatogr* **296** 293 *1984*.]

Interleukin-2 (recombinant human) *[94218-72-1]* **M_r ~15,000, amorphous.** Purified by reverse phase HPLC. [Weir and Sparks *Biochem J* **245** 85 *1987*; Robb et al. *Proc Natl Acad Sci USA* **81** 6486 *1984*.]

Interleukins (IL-1, IL-2 —IlL18]. Interleukins are cytokines which cause a variety of effects including stimulation of cell growth and proliferation of specific cells, e.g. stem cells, mast cells, activated T cells, colony stimulating factors etc, as well as stimulating other ILs, prostaglandins release etc. They are small glycosylated proteins (*ca* 15 kD, 130-180 amino acids produced from longer precursors) and are sometimes referred to by other abbreviations, e.g. IL-2 as TCGF (T cell growth factor), IL-3 as multi-CSF (multilineage colony stimulating factor, also as BPA, HCSF, MCSF and PSF). They are produced in very small amounts and are commercially made by recombinant DNA techniques in bacteria or Sf21 insect cells. Interleukins for human (h-IL), mouse (m-IL) and rat (r-IL) are available and up to IL-18 are available commercially in such purity that they can be used directly without further refinement, particularly those that have been obtained by recombinant DNA procedures which are specific. As well as the interleukins, a variety of antibodies for specific IL reactions are available for research or IL identification. [Symons et al. Lymphokines and Interferons, *A Practical Approach*, Clemens et al. Eds, p. 272 *1987*; IRL Press, Oxford, ISBN 1852210354, 1852210362; Thomson et al. Eds, *The Cytokine Handbook*, 3rd Edn, *1998*; Academic Press, San Diego, ISBN 0126896623.]

Iodonitrotetrazolium chloride (2[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyl-2*H*-tetrazolium chloride) *[146-68-9]* **M 505.7, m 229°(dec), ~245°(dec).** Recrystd. from H_2O, aqueous EtOH or EtOH-Et_2O. Alternatively dissolve in the minimum volume of EtOH and add Et_2O; or dissolve in hot H_2O (charcoal), filter and ppte by adding conc HCl. Filter solid off and dry at 100°. Solubility in H_2O at 25° is 0.5%, and in hot MeOH-H_2O (1:1) it is 5%. [Fox and Atkinson *J Am Chem Soc* **72** 3629 *1950*.]

Iodonitrotetrazolium violet-Formazan *[7781-49-9]* **M 471.3, m 185-186°.** Dissolve in boiling dioxane (20g in 300mL), add H_2O (100mL) slowly, cool, filter and dry *in vacuo* at 100°. Its solubility in $CHCl_3$ is ~1%. [UV: Fox and Atkinson *J Am Chem Soc* **72** 3629 *1950*.]

5-Iodouridine (5-iodo-1-[β-D-ribofuranosyl]-pyrimidine-2,4(1*H*)-dione) *[1024-99-3]* **M 370.1, m 205-208°(dec), 210-215°(dec), $[\alpha]_D^{20}$ -23.5° (c 1, H_2O), pK^{20} 8.5.** Recrystd from H_2O and dried *in vacuo* at 100°. UV has λ_{max} 289nm (0.01N HCl) and 278nm (0.01N NaOH). [Prusoff et al. *Cancer Res* **13** 221 *1953*.]

3-Isobutyl-1-methylxanthine (3-isobutyl-1-methylpurine-2,6-dione) *[28822-58-4]* **M 222.3, m 199-210°, 202-203°, pK_{Est} ~ 6.7 (acidic NH).** Recrystd from aqueous EtOH.

Isopentenyl pyrophosphate *[358-71-4]* **M 366.2, $pK_{Est(1)}$~<2, $pK_{Est(2)}$~<2, $pK_{Est(3)}$~3.95, $pK_{Est(4)}$~6.26.** Purified by chromatography on Whatman No 1 paper using *tert*-butyl alcohol/formic acid/water (20:5:8, R_F 0.60) or 1-propanol/ammonia/water (6:3:1. R_F 0.48). Also purified by chromatography on a DEAE-cellulose column or a Dowex-1 (formate form) ion-exchanger using formic acid and ammonium formate as eluents. A further purification step is to convert it to the monocyclohexylammonium salt by passage through a column of Dowex-50 (cyclohexylammonium form) ion-exchange resin. Can also be converted into its lithium salt.

DL-Isoserine (±-3-amino-2-hydroxypropionic acid) *[632-12-2]* **M 105.1, m 250-252°(dec), 235°(dec), 237°(dec), 245°(dec), pK_1^{25} 2.78 (acidic), pK_2^{25} 9.27 (basic).** Recrystd from H_2O or 50% aqueous EtOH. It has an isoelectric pH of 6.02. [Rinderknocht and Niemann *J Am Chem Soc* **75** 6322 *1953*; Gundermann and Holtmann *Chem Ber* **91** 160 *1958*; Emerson et al. *J Biol Chem* **92** 451 *1931*.] The *hydrobromide* has **m** 128-130° (from aqueous HBr) [Schöberl and Braun *Justus Liebigs Ann Chem* **542** 288 *1939*].

Isoxanthopterin (2-amino-4,7-dihydroxypteridine) *[529-69-1]* **M 179.4, m>300°, pK_1^{20} -0.5 (basic), pK_2^{20} 7.34 (acidic), pK_3^{20} 10.06 (acidic).** Purified by repeated pptn from alkaline solutions by acid (preferably AcOH), filter, wash well with H_2O then EtOH and dried at 100°. Purity is checked by paper chromatography [R_F 0.15 (*n*-BuOH, AcOH, H_2O, 4:1:1); 0.33 (3% aq NH_4OH). [Goto et al. *Arch Biochem*

Biophys **111** 8 *1965.*] For biochemistry see Blakley *Biochemistry of Folic Acid and Related Pteridines* North Holland Publ Co, Amsterdam 1969.]

Kanamycin B **(Bekanamycin, 4-*O*-[2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl]-6-*O*-[3-amino-3-deoxy-α-D-glucopyranosyl]-2-deoxystreptamine)** *[4696-76-8, 29701-07-3 (sulfate salt)]* **M 483.5, m 170-179°(dec), 178-182°(dec), $[\alpha]_D^{18}$ +130° (c 0.5, H_2O), pK 7.2.** A small quantity (24mg) can be purified on a small Dowex 1 x 2 column (6 x 50mm), the correct fraction is evapd to dryness and the residue crystd from EtOH containing a small amount of H_2O. [Umezawa et al. *Bull Chem Soc Jpn* **42** 537 *1969.*] It has been crystd from H_2O by dissolving ~1g in H_2O (3mL), adding Me_2NCHO (3mL) setting aside at 4° overnight, The needles are collected and dried to constant weight at 130°. It has also been recrystd from aq EtOH. It is slightly sol in $CHCl_3$ and isoPrOH. [IR: Wakazawa et al. *J Antibiot* **14A** 180, 187 *1961*; Ito et al. *J Antibiot* **17 A** 189 *1964.*]

Lactate dehydrogenase **(from dogfish, Beef muscle)** *[9001-60-9]* **M_r 140,000 [EC 1.1.1.27].** 40-Fold purification by affinity chromatography using Sepharose 4B coupled to 8-(6-aminohexyl)amino-5'-AMP or -NAD^+. [Lees et al. *Arch Biochem Biophys* **163** 561 *1974*; Pesce et al. *J Biol Chem* **239** 1753 *1964.*]

Lactoferrin (from human whey). Purified by direct adsorption on cellulose phosphate by batch extraction, then eluted by a stepped salt and pH gradient. [Foley and Bates *Anal Biochem* **162** 296 *1987.*]

Lecithin (1,2-diacylphosphatidylcholine mixture) *[8002-43-5]* **M ~600-800, amorphous.** From hen egg white. Purified by solvent extraction and chromatography on alumina. Suspended in distilled water and kept frozen until used [Lee and Hunt *J Am Chem Soc* **106** 7411 *1984*, Singleton et al. *J Am Oil Chem Soc* **42** 53 *1965*]. For purification of commercial egg lecithin see Pangborn [*J Biol Chem* **188** 471 *1951*].

Lectins (proteins and/or glycoproteins of non-immune origin that agglutinate cells, from seeds of *Robinia pseudoacacia*), M ~100,000. Purified by pptn with ammonium sulfate and dialysis; then chromatographed on DE-52 DEAE-cellulose anion-exchanger, hydroxylapatite and Sephacryl S-200. [Wantyghem et al. *Biochem J* **237** 483 *1986.*]

Leucopterin (2-amino-5,8-dihydropteridine-4,6,7(1*H*)-trione) *[492-11-5]* **M 195.1, m >300° (dec), pK_1^{20}-1.66, pK_2^{20} 7.56, pK_3^{20} 9.78, pK_4^{20} 13.6.** Purified by dissolving in aqueous NaOH, stirring with charcoal, filtering and precipitating by adding aqueous HCl, then drying at 100° in a vacuum. It separates with 0.5 moles of H_2O. Its solubility in H_2O is 1g/750 litres [Albert et al. *J Chem Soc* 4219 *1952*; Albert and Wood *J Appl Chem* (*London*) **2** 591 *1952*; Pfleiderer *Chem Ber* **90** 2631 *1957*].

DL-α-Lipoamide (±-6,8-thioctic acid amide, 5-[1,2]-dithiolan-3-ylvaleric acid amide) *[3206-73-3]* **M 205.3, m 124-126°, 126-129°, 130-131°.** Recrystd from EtOH and has UV with λ_{max} 331nm in MeOH. [Reed et al. *J Biol Chem* **232** 143 *1958*; IR: Wagner et al. *J Am Chem Soc* **78** 5079 *1956.*]

DL-α-Lipoic acid (±-6,8-thioctic acid, 5-[1,2]-dithiolan-3-ylvaleric acid) *[1077-28-7]* **M 206.3, m 59-61°, 60.5-61.5° and 62-63°, b 90°/10^{-4}mm, 150°/0.1mm, pK^{25} 4.7.** It forms yellow needles from cyclohexane or hexane and has been distd at high vacuum, and sublimes at ~90° and very high vacuum. Insoluble in H_2O but dissolves in alkaline soln. [Lewis and Raphael *J Chem Soc* 4263 *1962*; Soper et al. *J Am Chem Soc* **76** 4109; Reed and Niu *J Am Chem Soc* **77** 416 *1955*; Tsuji et al. *J Org Chem* **43** 3606 *1978*; Calvin *Fed Proc USA* **13** 703 *1954.*] The *S-benzylthiouronium salt* has **m** 153-154° (evacuated capillary; from MeOH), 132-134°, 135-137° (from EtOH). The *d-* and *l-* forms have **m** 45-47.5° and $[\alpha]_D^{23}$ ±113° (c 1.88, $^*C_6H_6$) and have UV in MeOH with λ_{max} at 330nm (ε 140).

Lipoprotein lipase (from bovine skimmed milk) *[9004-02-8]* **[EC 3.1.1.34].** Purified by affinity chromatography on heparin-Sepharose [Shirai et al. *Biochim Biophys Acta* **665** 504 *1981*].

Lipoproteins (from human plasma). Individual human plasma lipid peaks were removed from plasma by ultracentrifugation, then separated and purified by agarose-column chromatography. Fractions were characterised immunologically, chemically, electrophoretically and by electron microscopy. [Rudel et al. *Biochem J* **13** 89 *1974*.]

Lipoteichoic acids (from gram-positive bacteria) *[56411-57-5].* Extracted by hot phenol/water from disrupted cells. Nucleic acids that were also extracted were removed by treatment with nucleases. Nucleic resistant acids, proteins, polysaccharides and teichoic acids were separated from lipoteichoic acids by anion-exchange chromatography on DEAE-Sephacel or by hydrophobic interaction on octyl-Sepharose [Fischer et al. *Eur J Biochem* **133** 523 *1983*].

D-Luciferin (firefly luciferin, *S*-2[6-hydroxybenzothiazol-2-yl]-4,5-dihydrothiazol-4-carboxylic acid), *[2591-17-5]* **M 280.3, m 189.5-190°(dec), 196°(dec), 201-204°, 205-210°(dec, browning at 170°), $[\alpha]_D^{22}$ -36° (c 1.2, DMF), $pK_{Est(1)}$~ 1.2 (benzothiazole-N), $pK_{Est(2)}$~ 1.6 (thiazolidine-N), $pK_{Est(3)}$~ 6.0 (CO_2H), $pK_{Est(4)}$ 8.5 (6OH).** Recrystallises as pale yellow needles from H_2O, or MeOH (83mg from 7mL). It has UV λ_{max} at 263 and 327nm (log ε 3.88 and 4.27) in 95% EtOH. The Na salt has a solubility of 4mg in 1 mL of 0.05M glycine. [White et al. *J Am Chem Soc* **83** 2402 11961, **85** 337 *1963*; UV and IR: Bitler and McElroy *Arch Biochem* **72** 358 *1957*; Review: Cormier et al. *Fortschr Chem Org Naturst* **30** 1 *1973*.]

Lumiflavin (7,8,10-trimethylbenzo[*g*]pteridine-2,4(3*H*,10*H*)-dione) *[1088-56-8]* **M 256.3, m 330°(dec), 340°(dec), pK 10.2.** Forms orange crystals upon recrystn from 12% aqueous AcOH, or from formic acid. It sublimes at high vacuum. It is freely soluble in $CHCl_3$, but not very soluble in H_2O and most organic solvents. In H_2O and $CHCl_3$ soln it has a green fluorescence. UV has λ_{max} at 269, 355 and 445nm (ε 38,800, 11,700 and 11,800 respectively) in 0.1N NaOH and 264, 373 and 440nm (ε 34,700, 11,400 and 10,400 respectively) in 0.1N HCl while UV in $CHCl_3$ has λ_{max} at 270, 312, 341, 360, 420, 445 and 470nm. [Hemmerich et al. *Helv Chim Acta* **39** 1242 *1956*; Holiday and Stern *Chem Ber* **67** 1352 *1834*; Yoneda et al. *Chem Pharm Bull Jpn* **20** 1832 *1972*; Birch and Moye *J Chem Soc* 2622 *1958*; Fluorescence: Kuhn and Moruzzi *Chem Ber* **67** 888 *1934*.]

Magnesium protoporphyrin dimethyl ester *[14724-63-1]* M 580.7.

Crude product dissolved in as little hot dry $^*C_6H_6$ as possible and left overnight at room temperature to cryst. [Fuhrhop and Graniek *Biochem Prep* **13** 55 *1971*.]

α-Melanotropin *[581-05-5]* **(13 amino acids peptide), $[\alpha]_D^{25}$ -58.5° (c 0.4, 10% aq AcOH).** Extract separated by ion-exchange on carboxymethyl cellulose, desalted, evapd and lyophilised, then chromatographed on Sephadex G-25. [Lande et al. *Biochem Prep* **13** 45 *1971*.]

ß-Melanotropin. *[9034-42-8]* **(18-22 amino acids peptide), amorphous.** Extract separated by ion-exchange on carboxymethyl cellulose, desalted, evapd and lyophilised, then chromatographed on Sephadex G-25. [Lande et al. *Biochem Prep* **13** 45 *1971*.]

6-Mercaptopurine monohydrate *[6112-76-1]* **M 170.2, m 314-315°(dec), ~315°(dec), 313-315°(dec), pK_1^{20} 7.77, pK_2^{20} 10.84.** Recrystallises from H_2O as yellow crystals of the monohydrate which become anhydrous on drying at 140°. It has UV λ_{max} at 230 and 312nm (ε 14,000 and 19,600) in 0.1N NaOH; 222 and 327nm (ε 9,2400 and 21,300), and 216 and 329nm (ε 8,740 and 19,300) in MeOH. [Albert and Brown *J Chem Soc* 2060 *1954*; IR: Brown and Mason *J Chem Soc* 682 *1957*; UV: Fox et al. *J Am Chem Soc* **80** 1669 *1958*; UV: Mason *J Chem Soc* 2071 *1954*.]

6-Mercaptopurine-9-β-D-ribofuranoside *[574-25-4]* **M 284.3, m 208-210°(dec), 210-211°(dec), 220-223°(dec), 222-224°(dec), $[\alpha]_D^{25}$ -73° (c 1, 0.1N NaOH), pK 7.56.** Recrystd from H_2O or EtOH. It has UV λ_{max} in H_2O at 322nm (pH 1), 320 nm (pH 6.7) and 310nm (pH 13). [IR: Johnson et al. *J Am Chem Soc* **80** 699 *1958*; UV: Fox et al. *J Am Chem Soc* **80** 1669 *1958*.]

Metallothionein (from rabbit liver) *[9038-94-2].* Purified by precipitation to give Zn- and Cd-containing protein fractions and running on a Sephadex G-75 column, then isoelectric focusing to give two protein peaks [Nordberg et al. *Biochem J* **126** 491 *1972*].

Methadone hydrochloride (2-dimethylamino-4-ethoxycarbonyl-4,4-diphenylbutane HCl) *[1095-90-5]* **M 345.9, m 241-242°, pK_1^{25} 8.94, pK_2^{20} 10.12 (free base).** Crystd from EtOH.

Methoxantin coenzyme (PQQ, pyrrolo quinoline quinone, 2,7,9-tricarboxy-1*H*-pyrrolo-[2,3-*f*]-quinoline-4,5-dione, 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid) *[72909-34-3]* **M 330.2, m 220°(dec).** Efflorescent yellow-orange needles on recrystn from H_2O by addition of Me_2CO, or better from a supersaturated aqueous soln, as it forms an acetone adduct. [Forrest et al. *Nature* **280** 843 *1979*.] It has also been purified by passage through a C-18 reverse phase silica cartridge or a silanised silica gel column in aqueous soln whereby methoxantin remains behind as a red-orange band at the origin. This band is collected and washed thoroughly with dilute aqueous HCl (pH 2) and is then eluted with MeOH-H_2O (7:3) and evapd *in vacuo* to give the coenzyme as a red solid. It has also been purified by dissolving in aqueous 0.5M K_2CO_3 and acidified to pH 2.5 whereby PQQ pptes as a deep red solid which is collected and dried *in vacuo*. Methoxantin elutes at 3.55 retention volumes from a C18 μBondapak column using H_2O-MeOH (95:5) + 0.1% AcOH pH 4.5. It has UV λ_{max} at 247 and 330nm (shoulder at 270nm) in H_2O and λ_{max} at 250 and 340nm in H_2O at pH 2.5. With excitation at λ_{ex} 365nm it has a λ_{max} emission at 483nm. The ^{13}C NMR has δ: 113.86, 122.76, 125.97, 127.71, 130.68, 137.60, 144.63, 146.41, 147.62, 161.25, 165.48, 166.45, 173.30 and 180.00.

When a soln in 10% aqueous MeCO is adjusted to pH 9 with aqueous NH_3 and kept at 25° for 30 min, the *acetone adduct* is formed; UV has λ_{max} at 250, 317 and 360nm (H_2O, pH 5.5) and with λ_{ex} at 360nm it has max fluorescence at λ_{max} at 465nm; and the ^{13}C NMR [$(CD_3)_2SO$, TMS] has δ: 29.77, 51.06, 74.82, 111.96, 120.75, 121.13, 125.59, 126.88, 135.21, 139.19, 144.92, 161.01, 161.47, 165.17, 168.61, 190.16 and 207.03. It also forms a *methanol adduct*.

When it is reacted with Me_2SO_4-K_2CO_3 in dry Me_2NCHO at 80° for 4h, it forms the *trimethyl ester* which has **m** 265-267°(dec) [260-263°(dec)] after recrystn from hot MeCN (orange crystals) with UV λ_{max} at 252 and 344nm (H_2O) and 251, 321 and 373nm (in MeOH; MeOH adduct ?). [Duine et al. *Eur J Biochem* **108** 187 *1980*; Duine et al. *Adv Enzymology* **59** 169 *1987*; Corey and Tramontano *J Am Chem Soc* **103** 5599 *1981*; Gainor and Weinreb *J Org Chem* **46** 4319 *1981*; Hendrickson and de Vries *J Org Chem* **17** 1148 *1982*; McKenzie, Moody and Reese *J Chem Soc Chem Commun* 1372 *1983*.]

Methyl benzylpenicillinate *[653-89-4]* **M 348.3, m 97°, $[\alpha]_D^{20}$ +328° (c 1, MeOH).** Crystd from CCl_4.

5-Methylphenazinium methyl sulfate *[299-11-6]* **M 306.3, m 155-157° (198°dec by rapid heating).** It forms yellow prisms from EtOH (charcoal). Solubility in H_2O at 20° is 10%. In the presence of aqueous KI it forms a *semiquinone* which crystallises as blue leaflets from EtOH. [Wieland and Roseen *Chem Ber* **48** 1117 *1913*; Voriskova *Collect Czech Chem Commun 12* 607 *1947*; Bülow *Chem Ber* **57** 1431 *1924*.]

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) *[23007-85-4]* **M 209.7, m 196-198°, pK_{Est} ~ 9.3.** Purified by recrystn from Me_2CO + isoPrOH. The *free base* has **b** 137-142°/0.8 mm, n_D^{25} 1.5347. [Schmidle and Mansfield *J Am Chem Soc* **78** 425 *1956*; Defeudis *Drug Dev Res* **15** 1 *1988*.]

6-α-Methylprednisolone (Medrol, 11β,17-21-trihydroxy-6α-methylpregna-1,4-dien-3,20-dione) *[83-43-2]* **M 347.5, m 226-237°, 228-237°, 240-242°, $[\alpha]_D^{24}$ +91° (c 0.5, dioxane).** Recrystd from EtOAc. UV has λ_{max} in 95% EtOH 243nm (ε 14,875). The *21-acetoxy derivative* has **m** 205-

208° (from EtOAc), $[\alpha]_D^{24}$ +95° (c 1, $CHCl_3$). [Spero et al. *J Am Chem Soc* **78** 6213 *1956*; Fried et al. *J Am Chem Soc* **81** 1235 *1959*; ^1H NMR: Slomp and McGarvey *J Am Chem Soc* **81** 2200 *1959*.]

5-Methyltetrahydrofolic acid disodium salt (prefolic A) *[68792-52-9]* **M 503.4, pK_1 2.4 (N10 protonation), pK_2 2.7 (pyrimidine N1 protonation), pK_3 3.5 (α-CO_2H), pK_4 4.9 (γ-CO_2H), pK_5 5.6 (N5-Me) , pK_6 8.5 (3NHCO acidic).** Check purity by measuring UV at pH 7.0 (use phosphate buffer) and it should have λ_{max} 290nm and λ_{min} 245nm with a ratio of A_{290}/A_{250} of 3.7. This ratio goes down to 1.3 as oxidation to the dihydro derivative occurs. The latter can be reduced back to the tetrahydro compound by reaction with 2-mercaptoethanol at room temp. If oxidation had occurred then the compound should be chromatographed on DEAE-cellulose (~0.9 milliequiv/g, in AcO^- form) in $(NH_4)_2CO_3$ (1.5 M) and washed with 1M NH_4OAc containing 0.01M mercaptoethanol till free from UV absorption and then washed with 0.01M mercaptoethanol. All is done in a nitrogen atmosphere. The reduced folate is then eluted with a gradient between 0.01M mercaptoethanol and 1M NH_4OAc containing 0.01M mercaptoethanol and the fractions with absorption at 290nm are collected. These are evapd under reduced pressure at 25° and traces of NH_4OAc and H_2O are removed at high vacuum/25° (~24-48h). The residue is dissolved in the minimum volume of 0.01M mercaptoethanol and an equivalent of NaOH is added to convert the acid to the diNa salt and evaporated to dryness at high vacuum/25°. The product should have λ_{max} 290nm (ε 32,000) in pH 7.0 buffer. [Sakami *Biochem Prep* **10** 103 *1963*.]

5-Methyltryptamine hydrochloride (3-[2-aminoethyl]-5-methylindole hydrochloride) *[1010-95-3]* **M 210.7, m 289-291°(dec), 290-292°, $pK_{Est(1)}$~ -3 (protonation of ring NH), $pK_{Est(2)}$~ 9.0 (CH_2NH_2), $pK_{Est(3)}$~ 10.9 (acidic indole NH).** Recrystd from H_2O. The *free base* has **m** 93-95° (from $^*C_6H_6$-cyclohexane), and the *picrate* has **m** 243°(dec) (from EtOH). [Young *J Chem Soc* 3493 *1958*; Gaddum et al. *Quart J Exp Physiol* **40** 49 *1955*; Röhm *Hoppe Seyler's Z Physiol Chem* **297** 229 *1954*.]

4-Methylumbelliferone(β) hydrate (7-hydroxy-4-methylcoumarin) *[90-33-5]* **M 194.2, m 185-186°, 185-188°, 194-195°, pK_{Est} ~ 10.0 (phenolic OH).** Purified by recrystn from EtOH. It is insoluble in cold H_2O, slightly soluble in Et_2O and $CHCl_3$, but soluble in MeOH and AcOH. It has blue fluorescence in aqueous EtOH, and has UV λ_{max} 221, 251 and 322.5nm in MeOH. IR has ν 3077 br, 1667, 1592, 1385, 1267, 1156, 1130 and 1066 cm^{-1}. The *acetate* has **m** 153-154°. [Woods and Sapp *J Org Chem* **27** 3703 *1962*.]

4-Methylumbellifer-7-yl-α-D-glucopyranoside *[17833-43-1]* **M 338.3, m 221-222°, $[\alpha]_D^{20}$ 237° (c 3, H_2O).** Recrystd from hot H_2O.

4-Methylumbellifer-7-yl-β-D-glucopyranoside *[18997-57-4]* **M 338.3, m 210-212°, 211°, $[\alpha]_D^{20}$ -61.5° (c 2, pyridine), -89.5° (c 0.5, H_2O for half hydrate).** Recrystallises as the half hydrate from hot H_2O. [Constantzas and Kocourek *Collect Czech Chem Commun* **24** 1099 *1959*; De Re et al. *Ann Chim (Rome)* **49** 2089 *1959*.]

1-Methyluric acid *[708-79-2]* **M 182.1, m >350°, pK_1 5.75 (basic), pK_2 10.6 (acidic).** Recrystd from H_2O. [Bergmann and Dikstein *J Am Chem Soc* **77** 691 *1955*.] It has UV λ_{max} at 231 and 283. nm (pH 3) and 217.5 and 292.5nm (pH >12) [Johnson *Biochem J* **5** 133 *1952*].

Mevalonic acid lactone *[674-26-0]* **M 130.2, m 28°, b 145-150°/5mm.** Purified *via* the *dibenzylethylenediammonium salt* (**m** 124-125°) [Hofmann et al. *J Am Chem Soc* **79** 2316 *1957*], or by chromatography on paper or on Dowex-1 (formate) column. [Bloch et al. *J Biol Chem* **234** 2595 *1959*.] Stored as *N,N'*-dibenzylethylenediamine (DBED) salt, or as the lactone in a sealed container at 0°.

Mevalonic acid 5-phosphate *[1189-94-2]* **M 228.1, $pK_{Est(1)}$~ 1.5 (PO_4H_2), $pK_{Est(2)}$~ 4.4 (CO_2H), $pK_{Est(3)}$~ 6.31 (PO_4H^-).** Purified by conversion to the *tricyclohexylammonium salt* (**m** 154-156°) by treatment with cyclohexylamine. Crystd from water/acetone at -15°. Alternatively, the phosphate was chromatographed by ion-exchange or paper (Whatman No 1) in a system isobutyric acid/ammonia/water (66:3:30; R_F 0.42). Stored as the cyclohexylammonium salt.

Mevalonic acid 5-pyrophosphate *[1492-08-6]* **M 258.1, $pK_{Est(1)}$~<2, $pK_{Est(2)}$~<2, $pK_{Est(3)}$~3.95 (PO_4), $pK_{Est(4)}$ 4.4 (CO_2H), $pK_{Est(5)}$ ~6.26 (PO_4).** Purified by ion-exchange chromatography on Dowex-1 formate [Bloch et al. *J Biol Chem* **234** 2595 *1959*], DEAE-cellulose [Skilletar and Kekwick, *Anal Biochem* **20** 171 *1967*], on by paper chromatography [Rogers et al. *Biochem J* **99** 381 *1966*]. Likely impurities are ATP and mevalonic acid phosphate. Stored as a dry powder or as a slightly alkaline (pH 7-9) soln at -20°.

Mithramycin A (Aureolic acid, Plicamycin) *[18378-89-7]* **M 1085.2, m 180-183°, $[\alpha]_D^{20}$ -51° (c 0.3, EtOH), pK_{Est} ~ 9.2.** Purified from $CHCl_3$, and is soluble in MeOH, EtOH, Me_2CO, EtOAc, Me_2SO and H_2O, and moderately soluble in $CHCl_3$, but is slightly soluble in $^*C_6H_6$ and Et_2O. Fluorescent antitumour agent used in flow cytometry. [Thiem and Meyer *Tetrahedron* **37** 551 *1981*; NMR: Yu et al. *Nature* **218** 193 *1968*.]

Mitomycin C *[50-07-7]* **M 334.4, m >360°, $pK_{Est(2)}$~ 8.0.** Blue-violet crystals form $^*C_6H_6$-pet ether. It is soluble in Me_2CO, MeOH and H_2O, moderately soluble in $^*C_6H_6$, CCl_4 and Et_2O but insoluble in pet ether. It has UV λ_{max} at 216, 360 and a weak peak at 560nm in MeOH. [Stevens et al. *J Med Chem* **8** 1 *1965*; Shirahata and Hirayama *J Am Chem Soc* **105** 7199 *1983*.]

Muramic acid [*R*-2(2-amino-2-deoxy-D-glucose-3-yloxy)-propionic acid] *[1114-41-6]* **M 251.2, m 145-150°(dec), 152-154°(dec), 155°(dec), $[\alpha]_D^{25}$ +109° (c 2, H_2O), +165.0° (extrapolated to 0 time) → +123° [after 3h (c 3, H_2O)], $pK_{Est(1)}$~ 3.8 (CO_2), $pK_{Est(2)}$~ 7.7 (NH_2).** It has been recrystd from H_2O or aqueous EtOH as monohydrate which loses H_2O at 80° *in vacuo* over P_2O_5. Sometimes contains some NaCl. It has been purified by dissolving 3.2g in MeOH (75mL), filtered from some insoluble material, concentrated to ~10mL and refrigerated. The colourless crystals are washed with absolute MeOH. This process does not remove NaCl; to do so the product is recrystd from a equal weight of H_2O to give a low yield of very pure acid (0.12g). On paper chromatography 0.26μg give one ninhydrin positive spot after development with 75% phenol (R_F 0.51) or with *sec*-BuOH-HCO_2O-H_2O (7:1:2) (R_F 0.30). [Matsushima and Park *Biochem Prep* **10** 109 *1963*; *J Org Chem* **27** 3581 *1962*.] The acid has been also purified by dissolving 990mg in 50% aqueous EtOH (2mL), cooling, collecting the colourless needles on a sintered glass funnel and dried over P_2O_5 at 80°/0.1mm to give the anhydrous acid. [Lambert and Zilliken *Chem Ber* **93** 2915 *1960*.] Alternatively the acid is dissolved in a small volume of H_2O, neutralised to pH 7 with ion exchange resin beads (IR4B in OH^- form), filtered, evaporated and dried. The residue is recrystd from 90% EtOH (v/v) and dried as above for 24h. [Strange and Kent *Biochem J* **71** 333 *1959*.] The *N-acetyl* derivative has **m** ~125° (dec) and $[\alpha]_D^{20}$ +41.2° after 24h (c 1.5, H_2O). [Watanabe and Saito *J Bacteriol* **144** 428 *1980*.]

Muscimol (pantherine, 5-aminoethyl-3[2*h*]-isoxazolone) *[2763-96-4]* **M 114.1, m 170-172°(dec), 172-174°(dec), 172-175°, 175°, 176-178°(dec), $pK_{Est(1)}$~ 6 (acidic, ring 2-NH), $pK_{Est(2)}$~ 8 ($CH_2CH_2NH_2$).** Recrystd from MeOH-tetrahydrofuran or EtOH and sublimed at 110-140° (bath) at 10^{-4} mm and gives a yellow spot with ninhydrin which slowly turns purple [NMR: Bowden et al. *J Chem Soc (C)* 172 *1968*]. Also purified by dissolving in the minimum volume of hot H_2O and adding EtOH dropwise until cloudy, cool, and colourless crystals separate; IR: ν 3445w, 3000-2560w br, 2156w, 1635s and 1475s cm^{-1}. [NMR: Jager and Frey *Justus Liebigs Ann Chem* 817 1982.] Alternatively it has been purified by two successive chromatographic treatments on Dowex 1 x 8 with the first elution with 2M AcOH and a second with a linear gradient between 0—2M AcOH and evaporating the desired fractions and recrystallising the residue from MeOH. [McCarry and Savard *Tetrahedron Lett* **22** 5153 *1981*; Nakamura *Chem Pharm Bull Jpn* **19** 46 *1971*.]

Mycophenolic acid (6-[1,3-dihydro-7-hydroxy-5-methoxy-4-methyl-1-oxoisobenzofuran-6-yl]-4-methylhex-4-enoic acid) *[24280-93-1]* **M 320.3, m 141°, 141-143°, $pK_{Est(1)}$~ 2.5 (CO_2H), $pK_{Est(2)}$~ 9.5 (phenolic OH).** Purified by dissolving in the minimum volume of EtOAc, applying to a silica gel column (0.05-0.2 mesh) and eluting with a mixture of EtOAc + $CHCl_3$ + AcOH (45:55:1) followed by recrystn from heptane-EtOAc, from aqueous EtOH or from hot H_2O and drying *in vacuo*. It is a weak dibasic acid moderately soluble in Et_2O, $CHCl_3$ and hot H_2O but weakly soluble in $^*C_6H_6$ and

toluene. [Birch and Wright *Aust J Chem* **22** 2635 *1969*; Canonica et al. *J Chem Soc Perkin Trans 1* 2639 *1972*; Birkinshaw, Raistrick and Ross *Biochem J* **50** 630 *1952*.]

Myoglobin (from sperm whale muscle). *[9047-17-0]* **M_r ~17,000.** Purified by CM-cellulose chromatography and Sephadex G-50 followed by chromatography on Amberlite IRC-50 Type III or BioRex 70 (<400mesh). The crystalline product as a paste in saturated $(NH_4)_2SO_4$ at pH 6.5-7.0 may be stored at 4° for at least 4 years unchanged, but must not be kept in a freezer. [Anres and Atassi *Biochemistry* **12** 942 *1980*; Edmundson *Biochem Prep* **12** 41 *1968*.]

Myricetin (Cannabiscetin, 3,3',4',5,5',7-hexahydroxyflavone) *[529-44-2]* **M 318.2, m >300°, 357°(dec) (polyphenolic pK_{Est}~8-11).** Recrystd from aq EtOH (**m** 357° dec, as monohydrate) or Me_2CO (**m** 350° dec, with one mol of Me_2CO) as yellow crystals. Almost insol in $CHCl_3$ and AcOH. The *hexaacetate* has **m** 213°. [Hergert *J Org Chem* **21** 534 *1956*; Spada and Cameroni *Gazzetta* **86** 965, 975 *1956*; Kalff and Robinson *J Chem Soc* **127** 181 *1925*.]

Nalidixic acid

(1-ethyl-7-methyl-1,8-naphthyridin-4-one-3-carboxylic acid) *[389-08-2]* **M 232.3, m 226.8-230.2°, 228-230°, 229-230°, pK 6.0.** Crystd from H_2O or EtOH as a pale buff powder. It is soluble at 23° in $CHCl_3$ (3.5%), toluene (0.16%), MeOH (0.13%), EtOH (0.09%), H_2O (0.01% and Et_2O (0.01%). It inhibits nucleic acid and protein synthesis in yeast. [Lesher et al. *J Med and Pharm Chem* **5** 1063 *1962*.]

Naloxone hydrochloride hydrate (Narcan, 1-*N*-propenyl-7,8-dihydro-14-hydroxymorphinan-6-one hydrochloride) *[51481-60-8]* **M 399.9, m 200-205°, $[\alpha]_D^{20}$ -164° (c 2.5, H_2O), $pK_{Est(1)}$~6 (N-propenyl), $pK_{Est(2)}$~ 9.6 (phenolic OH).** This opiate antagonist has been recrystd from EtOH + Et_2O or H_2O. It is soluble in H_2O (5%) and EtOH but insoluble in Et_2O. The *free base* has **m** 184° (177-178°) after recrystn from EtOAc, $[\alpha]_D^{20}$ -194.5° (c 0.93, $CHCl_3$). [Olofson et al. *Tetrahedron Lett* 1567 *1977*; Gold et al. *Med Res Rev* **2** 211 *1982*.]

Naltrexone hydrochloride dihydrate (1-*N*-cyclopropylmethyl-7,8-dihydro-14-hydroxy-morphinan-6-one hydrochloride) *[16676-29-2]* **M 413.9, m 274-276°, $[\alpha]_D^{20}$ -173° (c 1, H_2O), $pK_{Est(1)}$~ 6 (N-cyclopropylmethyl), $pK_{Est(2)}$~ 9.6 (phenolic OH).** This narcotic antagonist has been purified by recrystn from MeOH and dried air. The *free base* has **m** 168-170° after recrystn from Me_2CO. [Cone et al. *J Pharm Sci* **64** 618 *1975*; Gold et al. *Med Res Rev* **2** 211 *1982*.]

α-Naphthoflavone (7,8-benzoflavone) *[604-59-1]* **M 272.3, m 153-155°, 155°, pK 8-9 (phenolic OH).** Recrystd from EtOH or aqueous EtOH. [IR: Cramer and Windel *Chem Ber* **89** 354 *1956;* UV Pillon and Massicot *Bull Soc Chim Fr* 26 *1954*; Smith *J Chem Soc* 542 *1946*; Mahal and Venkataraman *J Chem Soc* 1767 *1934*.] It is a competitive inhibitor of human estrogen synthase. [Kellis and Vickery *Science* **225** 1032 *1984*.]

Naphthol AS-acetate (3-acetoxynaphthoic acid anilide) *[1163-67-3]* **M 305.3, m 152°, 160°.** Recrystd from hot MeOH and dried *in vacuo* over P_2O_5. It is slightly soluble in AcOH, EtOH, $CHCl_3$ or $^*C_6H_6$. It is a fluorogenic substrate for albumin esterase activity. [Chen and Scott *Anal Lett* **17** 857 *1984*.] At λ_{ex} 320nm it had fluorescence at λ_{em} 500nm. [Brass and Sommer *Chem Ber* **61** 1000 *1928*.]

1-Naphthyl phosphate disodium salt *[2183-17-7]* **M 268.1, pK_1^{26} 0.97, pK_2^{26} 5.85 (for free acid).** Purified through an acid ion-exchange column (in H^+ form) to give the *free acid* which is obtained by freeze drying and recrystn from Me_2CO + $^*C_6H_6$, or by adding 2.5 vols of hot $CHCl_3$ to a hot soln of 1 part acid and 1.2 parts Me_2CO and cooling (**m** 155-157°, 157-158°). The acid is dissolved in the minimum volume of H_2O to which 2 equivalents of NaOH are added and then freeze dried, or by adding the equivalent amount of MeONa in MeOH to a soln of the acid in MeOH and collecting the Na salt, washing with cold MeOH then Et_2O and drying in a vacuum. [Friedman and Seligman *J Am Chem Soc* **72** 624 *1950*; Chanley and Feageson *J*

Am Chem Soc **77** 4002 *1955*.] It is a substrate for alkaline phosphatase [Gomori *Methods Enzymol* **4** 381 *1957*, **128** 212 *1968*], and prostatic phosphatase [Babson *Clin Chem* **30** 1418 *1984*]. See entry on p. 444.

2-Naphthyl phosphate monosodium salt *[14463-68-4]* **M 246.2, m 296° (sintering at 228°), pK_1^{26} 1.28, pK_2^{26} 5.53 (for free acid).** The *free acid* is purified as for the preceding 1-isomer and has **m** 176-177°, 177-178° after recrystn from $CHCl_3$ + Me_2CO as the 1-isomer above. It is neutralised with one equivalent of NaOH and freeze dried or prepared as the 1-isomer above. Its solubility in H_2O is 5%. It also forms a 0.5 Na.1 H_2O salt which has **m** 203-205° (244° ?). [Friedman and Seligman *J Am Chem Soc* **72** 624 *1950*; Chanley and Fegeason *J Am Chem Soc* **77** 4002 *1955*.] See entry on p. 444 in Chapter 5.

D(+)-Neopterin *[2009-64-5]* **M 253.2, m >300°(dec), $[\alpha]_{546}^{20}$ +64.5° (c 0.14, 0.1M HCl), $[\alpha]_D^{25}$ +50.1° (c 0.3, 0.1N HCl), pK_1 2.23 (basic), pK_2 7.89 (acidic).** Purified as biopterin. Also purified on a Dowex 1 x 8 (formate form) column and eluted with 0.03M ammonium formate buffer pH 8.0 then pH 7.2. The fluorescent neopterin fraction is evapd under reduced pressure leaving neopterin and ammonium formate (the latter can be sublimed out at high vacuum). The residue is stirred for 24h with EtOH and the solid is collected and recrystd from H_2O [Viscontini et al. *Helv Chim Acta* **53** 1202 *1970*; see Wachter et al. Eds *Neopterin* W de Guyter, Berlin *1992*].

β-Nicotinamide adenine dinucleotide (diphosphopyridine nucleotide, NAD, DPN) *[53-84-9]* **M 663.4, $[\alpha]_D^{23}$ -34.8° (c 1, H_2O), pK_1 2.2 (PO_4H), pK_2 4.0 (adenine NH_2), pK_3 6.1 (PO_4^-).** Purified by paper chromatography or better on a Dowex-1 ion-exchange resin. The column was prepared by washing with 3M HCl until free of material absorbing at 260nm, then with water, 2M sodium formate until free of chloride ions and, finally, with water. NAD, as a 0.2% soln in water, adjusted with NaOH to pH 8, was adsorbed on the column, washed with water, and eluted with 0.1M formic acid. Fractions with strong absorption at 360nm were combined, acidified to pH 2.0 with 2M HCl, and cold acetone (*ca* 5L/g of NAD) was added slowly and with constant agitation. It was left overnight in the cold, then the ppte was collected in a centrifuge, washed with pure acetone and dried under vacuum over $CaCl_2$ and paraffin wax shavings [Kornberg *Methods Enzymol* **3** 876 *1957*]. Purified by anion-exchange chromatography [Dalziel and Dickinson *Biochemical Preparations* **11** 84 *1966*.] The purity is checked by reduction to NADH (with EtOH and yeast alcohol dehydrogenase) which has ε_{340mn} 6220 $M^{-1}cm^{-1}$. [Todd et al. *J Chem Soc* 3727,3733 *1957*.] [pKa, Lamborg et al. *J Biol Chem* **231** 685 *1958*.] The *free acid* crystallises from aq Me_2CO with $3H_2O$ and has **m** 140-142°. It is stable in cold neutral aqueous solns in a desiccator ($CaCl_2$) at 25°, but decomposes at strong acid and alkaline pH. Its purity is checked by reduction with yeast alcohol dehydrogenase and EtOH to NADH and noting the OD at 340nm. Pure NADH has ε_{340} 6.2 x $10^4 M^{-1}cm^{-1}$, i.e. 0.1μmole of NADH in 3mL and in a 1cm path length cell has an OD at 340nm of 0.207.

β-Nicotinamide adenine dinucleotide reduced di-Na salt trihydrate (reduced diphosphopyridine nucleotide sodium salt, NADH) *[606-68-8]* **M 763.5, pK as for NAD.** This coenzyme is available in high purity and it is advised to buy a fresh preparation rather than to purify an old sample as purification will invariably lead to a more impure sample contaminated with the oxidised form (NAD). It has UV λ_{max} at 340nm (ε 6,200 $M^{-1}cm^{-1}$) at which wavelength the oxidised form NAD has no absorption. At 340 nm a 0.161mM solution in a 1cm (pathlength) cell has an absorbance of 1.0 unit. The purity is best checked by the ratio A_{280nm}/A_{340nm} ~2.1, a value which increases as oxidation proceeds. The dry powder is stable indefinitely at -20°. Solutions in aqueous buffers at pH ~7 are stable for extended periods at -20° and for at least 8h at 0°, but are oxidised more rapidly at 4° in a cold room (e.g. almost completely oxidised overnight at 4°). [UV: Drabkin *J Biol Chem* **175** 563 *1945*; Fluorescence: Boyer and Thorell *Acta Chem Scand* **10** 447 *1956*; Redox: Rodkey *J Biol Chem* **234** 188 *1959*; Schlenk in *The Enzymes* **2** 250, 268 *1951*; Kaplan in *The Enzymes* **3** 105, 112 *1960*.] Deuterated NADH, i.e. NADD, has been purified through the anion exchange resin AG-1 x 8 (100-200 mesh, formate form) and through a Bio-Gel P-2 column. [Viola, Cook and Cleland *Anal Biochem* **96** 334 *1979*.]

β-Nicotinamide adenine dinucleotide phosphate (NADP, TPN) *[53-59-8]* **M 743.4, pK_1 1.1 (PO_4H_2), pK_2 4.0 (adenine NH_2), pK_3 6.1 (PO_4^-).** Purified by anion-exchange chromatography in much the same way as for NAD [Dalziel and Dickinson *Biochem J* **95** 311 *1965*; *Biochemical Preparations* **11** 87 *1966*]. Finally it is purified by dissolving in H_2O and precipitating with 4 volumes of Me_2CO and dried *in*

vacuo over P_2O_5. It is unchanged by storing *in vacuo* at 2°. [Hughes et al. *J Chem Soc* 3733 *1957*, Schuster and Kaplan *J Biol Chem* **215** 183 *1955*.] Deuterated NADPH, i.e. NADPD, has been purified through the anion exchange resin AG-1 x 8 (100-200 mesh, formate form) and through a Bio-Gel P-2 column. λ_{min} 259nm (ε 18.000) at pH 7.0. [Viola, Cook and Cleland *Anal Biochem* **96** 334 *1979*.]

β-Nicotinamide adenine dinucleotide phosphate reduced tetrasodium salt (reduced diphosphopyridine nucleotide phosphate sodium salt, NADPH) *[2646-71-1]* **M 833.4, pK as for NADP.** Mostly similar to NADH above.

β-Nicotinamide mononucleotide (NMN) *[1094-61-7]* **M 334.2, $[\alpha]_D^{23}$ -38.3° (c 1, H_2O), pK_{Est} ~ 6.1 (PO_4^-).** Purified by passage through a Dowex 1 (Cl^- form), washed with H_2O until no absorbance at 260 nm. The tubes containing NMN are pooled, adjusted to pH 5.5-6 and evapd *in vacuo* to a small volume. This is adjusted to pH 3 with dilute HNO_3 in an ice bath and treated with 20 volumes of Me_2CO at 0-5°. The heavy white ppte is collected by centrifugation at 0°. It is best stored wet and frozen or can be dried to give a gummy residue. It has λ_{max} 266nm (ε 4600) and λ_{min} 249nm (ε 3600) at pH 7.0 (i.e. no absorption at 340nm). It can be estimated by reaction with CN^- or hydrosulfite which form the 4-adducts equivalent to NADH) which has UV λ_{max} 340nm (ε 6200). Thus after reaction an OD_{340} of one is obtained from a 0.1612mM soln in a 1cm path cuvette. [Plaut and Plaut *Biochem Prep* **5** 56 *1957*; Maplan and Stolzenbach *Methods Enzymol* **3** 899 *1957*; Kaplan et al. *J Am Chem Soc* **77** 815 *1955*.]

(-)-Nicotine (1-methyl-2[3-pyridyl]-pyrrolidine) *[54-11-5]* **M 162.2, b 123-125°/17mm, 246.1°/730.5mm, 243-248°/atm (partial dec), d_4^{20} 1.097, n_D^{20} 1.5280, $[\alpha]_D^{20}$ -169° (c 1, Me_2CO), pK_1^{15} 6.16 (pyridine N^+), pK_2^{15} 10.96 (pyrrolidine N^+).** Very pale yellow *hygroscopic* oil with a characteristic odour (tobacco extract) with browns in air on exposure to light. Purifed by fractional distn under reduced pressure in an inert atmosphere. A freshly distd sample should be stored in dark sealed containers under N_2. It is a strong base, at 0.05 M soln it has a pH of 10.2. Very soluble in organic solvents. It is soluble in H_2O and readily forms salts. [UV: Parvis *J Chem Soc* **97** 1035 *1910*; Dobbie and Fox *J Chem Soc* **103** 1194 *1913*.] The *hydrochlorides* (mono- and di-) form deliquescent crystals soluble in H_2O and EtOH but insoluble in Et_2O. It has also been purified *via* the $ZnCl_2$ double salt. [Ratz *Monatsh Chem* **26** 1241 *1905*; Biosynthesis: Nakan and Hitchinson *J Org Chem* **43** 3922 *1978*.] The *picrate* has **m** 218° (from EtOH). **POISONOUS.**

(±)-Nicotine *[22083-74-5]* **M 162.2, b 242.3°/atm, d_4^{20} 1.082 (pK see above).** Purified by distn. Its solubility in EtOH is 5%. The *picrate* forms yellow needles from hot H_2O and has **m** 218°. The *methiodide* has **m** 219° (from MeOH).

Nisin *[1414-45-5]* **M 3354.2.** Polypeptide from *S. lactis*. Crystd from EtOH. [Berridge et al. *Biochem J* **52** 529 *1952*; synthesis by Fukase et al. *Tetrahedron Lett* **29** 795 *1988*.]

2-Nitrophenyl-β-D-galactopyranoside *[369-07-3]* **M 301.3, m 185-190°, 193°, 193-194°, $[\alpha]_D^{18}$ -51.9° (c 1, H_2O).** Purified by recrystn from EtOH. [Seidman and Link *J Am Chem Soc* **72** 4324 *1950*; Snyder and Link *J Am Chem Soc* **75** 1758 *1953*]. It is a chromogenic substrate for β-galactosidases [Jagota et al. *J Food Sci* **46** 161 *1981*].

4-Nitrophenyl-α-D-galactopyranoside *[7493-95-0]* **M 301.3, m 166-169°, 173°, $[\alpha]_D^{25}$ +248 (c 1, H_2O).** Purified by recrystn from H_2O or aqueous EtOH. The *monohydrate* has **m** 85° which resolidifies and melts at 151-152° (the hemihydrate) which resolidifies and melts again at 173° as the anhydrous form. Drying the monohydrate at 60° yields the hemihydrate and drying at 100° gives the anhydrous compound. The *tetraacetate* has **m** 147° after drying at 100°. [Jermyn *Aust J Chem* **15** 569 *1962*; Helfreich and Jung *Justus Liebigs Ann Chem* **589** 77 *1954*.] It is a substrate for α-galactosidase [Dangelmaier and Holmsen *Anal Biochem* **104** 182 *1980*].

4-Nitrophenyl-β-D-galactopyranoside *[3150-24-1]* **M 301.3, m 178°, 178-181°,181-182°, $[\alpha]_D^{20}$ -83° (c 1, H_2O).** Purified by recrystn from EtOH. [Horikoshi *J Biochem (Tokyo)* **35** 39 *1042*;

Goebel and Avery *J Exptl Medicine* **50** 521 *1929*; Snyder and Link *J Am Chem Soc* **75** 1758.] It is a chromogenic substrate for β-galactosidases [Buoncore et al. *J Appl Biochem* **2** 390 *1980*].

4-Nitrophenyl-α-D-glucopyranoside *[3767-28-0]* **M 301.3, m 206-212°, 216-217° (sinters at 210°), $[\alpha]_D^{20}$ +215° (c 1, H_2O).** Purified by recrystn from H_2O, MeOH or EtOH. [Jermyn *Aust J Chem* **7** 202 *1954*; Montgomery et al. *J Am Chem Soc* **64** 690 *1942*.] It is a chromogenic substrate from α-glucosidases [Oliviera et al. *Anal Biochem* **113** 188*1981*], and is a substrate for glucansucrases [Binder and Robyt *Carbohydr Res* **124** 287*1983*]. It is a chromogenic substrate for β-glucosidases [Weber and Fink *J Biol Chem* **255** 9030 *1980*].

4-Nitrophenyl-β-D-glucopyranoside *[2492-87-7]* **M 301.2, m 164°, 164-165°, 165°, $[\alpha]_D^{20}$ -107° (c 1, H_2O).** Purified by recrystn from EtOH or H_2O. [Montgomery et al. *J Am Chem Soc* **64** 690 *1942*; Snyder and Link *J Am Chem Soc* **75** 1758 *1953*.]

Nonactin *[6833-84-7]* **M 737.0, m 147-148°, $[\alpha]_D^{20}$ 0° (±2°) (c 1.2, $CHCl_3$).** This macrotetrolide antibiotic was rerystd from MeOH as colourless needles, and dries at 90°/20h/high vacuum. [*Helv Chim Acta* **38** 1445 *1955*, **55** 1371 *1972*; *Tetrahedron Lett* 3391 *1975*.]

***N*-Nonanoyl-*n*-methylglucamine (Mega-9)** *[85261-19-4]* **M 335.4, m 87-89°.** A non-ionic detergent purified as *n*-decanoyl-*N*-methylglucamine above. [Hildreth *Biochem J* **207** 363 *1982*.]

Nonyl-β-D-glucopyranoside *[69984-73-2]* **M 306.4, m 67.5-70°, $[\alpha]_D^{20}$ -34.4° (c 5, H_2O), $[\alpha]_D^{25}$ -28.8° (c 1, MeOH).** Purified by recrystn from Me_2CO and stored in well stoppered containers as it is *hygroscopic*. [Pigman and Richtmyer *J Am Chem Soc* **64** 369 *1942*.] It is a UV transparent non-ionic detergent for solubilising membrane proteins [Schwendener et al. *Biochem Biophys Res Commun* **100** 1055 *1981*.]

L-Noradrenaline (Adrenor, *R*-2-amino-1-[3,4-dihydroxyphenyl]ethan-1-ol, L-norepinephrine) *[51-41-2, 69815-49-2 (bitartrate salt)]* **M 169.2, m 216.5-218°(dec), ~220-230°(dec), $[\alpha]_D^{20}$ -45° (c 5, N HCl), $[\alpha]_D^{25}$ 37.3° (c 5, 1 equiv aqueous HCl), pK_1^{25} 5.58 (phenolic OH), pK_2^{25} 8.90 (phenolic OH), pK_3^{25} 9.78 (NH_2).** Recrystd from EtOH and stored in the dark under N_2. [pKa, Lewis *Brit J Pharmacol Chemother* **9** 488 *1954*; UV: Bergström et al. *Acta Physiol Scand* **20** 101 *1950*; Fluorescence: Bowman et al. *Science NY* **122** 32 *1955*; Tullar *J Am Chem Soc* **70** 2067 *1948*.] The *L-tartrate salt monohydrate* has **m** 102-104.5°, $[\alpha]_D^{25}$ -11° (c 1.6, H_2O), after recrystn from H_2O or EtOH.

L-Noradrenaline hydrochloride (Arterenol) *[329-56-6]* **M 205.6, m 145.2-146.4°, ~150°(dec), $[\alpha]_D^{25}$ -40° (c 6, H_2O), pK see above.** Recrystd from isoPrOH and stored in the dark as it is oxidised in the presence of light (see preceding entry). [Tullar *J Am Chem Soc* **70** 2067 *1948*.]

Novobiocin (7-[*O*³-carbamoyl-5-*O*⁴-dimethyl-β-L-*lyso*-6-desoxyhexahydropyranosyloxy]-4-hydroxy-3[4-hydroxy-3-{3-methylbut-2-enyl}-benzyl-amino]-8-methylcoumarin) *[303-81-1]* **M 612.6, two forms m 152-156° and m 172-174°, 174-178°, λ_{max} at 330nm (acid EtOH), 305nm (alk EtOH), $[\alpha]_D^{25}$ -63° (c 1, EtOH), pK_1 4.03 (4.2), pK_2 9.16.** Crystd from EtOH and stored in the dark. It has also been recrystd from Me_2CO-H_2O. [Hoeksema et al. *J Am Chem Soc* **77** 6710 *1955*; Kaczka et al. *J Am Chem Soc* **77** 9404 *1955*.]
The **sodium salt** *[1476-53-5]* **M 634.6, m 210-215°, 215-220°(dec), 222-229°, $[\alpha]_D^{25}$ -38° (c 1, H_2O)** has been recrystd from MeOH, then dried at 60°/0.5mm. [Sensi, Gallo and Chiesa, *Anal Chem* **29** 1611 *1957*; Kaczka et al. *J Am Chem Soc* **78** 4126 *1956*.]

5'-Nucleotidase (from Electric ray, *Torpedo sp*) *[9027-73-0]* **[EC 3.1.3.5], amorphous.** Purified by dissolving in Triton X-100 and deoxycholate, and by affinity chromatography on concanavalin A-Sepharose and AMP-Sepharose [Grondal and Zimmerman *Biochem J* **245** 805 *1987*].

Nucleotide thiophosphate analogues. The preparation and purification of [³H]ATPγS, [³H]GTPγS, s^6ITPγS (6-thioinosine), cl^6ITPγS (6-chloroinosine) and [³H]ATPγS are described and the general purification

was achieved by chromatography of the nucleotide thiophosphates in the minimum volume of H_2O placed onto a DEAE-Sephadex A25 column and eluting with a linear gradient of triethylammonium bicarbonate (0.1 to 0.6M for G and I nucleotides and 0.2 to 0.5M for A nucleotides). [*Biochim Biophys Acta* **276** 155 *1972.*]

Nystatin dihydrate (Mycostatin, Fungicidin) *[1400-61-9]* **M 962.1, m dec>160° (without melting by 250°), $[\alpha]_D^{25}$ -7° (0.1N HCl in MeOH), -10° (AcOH), +12° (Me_2NCHO), +21° (pyridine).** Light yellow powder with the following solubilities at ~28°: MeOH (1.1%), ethylene glycol (0.9%), H_2O (0.4%), CCl_4 (0.12%), EtOH (0.12%), $CHCl_3$ (0.05%) and $^*C_6H_6$ (0.03%). Could be ppted from MeOH soln by addition of H_2O. Aqueous suspensions of this macrolide antifungal antibiotic are stable at 100°/10min at pH 7.0 but decomposes rapidly at pH <2 and >9, and in the presence of light and O_2. [Birch et al. *Tetrahedron Lett* 1491, 1485 *1964*; Weiss et al. *Antibiot Chemother* **7** 374 *1957.*] It contains a mixture of components A_1, A_2 and A_3.

Octyl-β-D-glucopyranoside

[29836-26-8] **M 292.4, m 62-65°, 63.8-65°, $[\alpha]_D^{20}$ -34° (c 4, H_2O).** Purified by recrystn from Me_2CO. It is *hygroscopic* and should be stored in a well stoppered container. [Noller and Rockwell *J Am Chem Soc* **60** 2076 *1938*; Pigman and Richtmyer *J Am Chem Soc* **64** 369 *1942.*] It is a UV transparent non-ionic dialysable detergent for solubilising membrane proteins. The *α–D-isomer* with $[\alpha]_D^{20}$ +118° (c 1, MeOH) has similar solubilising properties. [Lazo and Quinn *Anal Biochem* **102** 68 *1980*; Stubbs et al. *Biochim Biophys Acta* **426** 46 *1976.*]

Orcine monohydrate (3,5-dihydroxytoluene) *[6153-39-5]* **M 142.2, m 56°, 56-58°, 58°, b 147°/5 mm, pK_1^{20} 9.48 (9.26), pK_2^{20} 11.20 (11.66).** Purified by recrystn from H_2O as the monohydrate. It sublimes *in vacuo* and the *anhydrous* compound has **m** 106.5-108° (110°, 108°). Also can be recrystd from $CHCl_3$ (plates) or $^*C_6H_6$ (needles or prisms). [UV: Kiss et al. *Bull Soc Chim Fr* 275 *1949*; Adams et al. *J Am Chem Soc* **62** 732 *1940.*]

Orosomucoid (glycoprotein α_1 acid, from human plasma) *[66455-27-4]* **M_r 42000-44000, amorphous.** Purified by passage through a carboxymethyl cellulose column and through a Sephadex G-25 column. [Aronson et al. *J Biol Chem* **243** 4564 *1968.*]

Orotic acid Li salt H_2O (1-carboxy-4,6-dihydroxypyrimidine Li salt H_2O) *[5266-20-6]* **M 180.0, m >300°, pK_1 2.8 (CO_2H), pK_2 9.4 (OH), pK_3 >13 (OH) (for free acid).** It is soluble in H_2O at 17° and 100°. Best to acidify an aqueous soln, isolating the free acid which is recrystd from H_2O (as monohydrate) **m** 345-347° (345-346°), then dissolving in EtOH, adding an equivalent amount of LiOH in EtOH and evaporating. Its solubility in H_2O is 1.28% (17°) and 2.34% (100°). [Bachstez *Chem Ber* **63** 1000 *1930*; Johnson and Shroeder *J Am Chem Soc* **54** 2941 *1932*; UV: Shugar and Fox *Biochim Biophys Acta* **9** 199 *1952.*]

Oxacillin sodium salt (5-methyl-3-phenyl-4-isoxazolylpenicillin sodium salt) *[1173-88-2]* **M 423.4, m 188°(dec), $[\alpha]_D^{20}$ +29° (c 1, H_2O), pK_{Est} ~ 2.7.** This antibiotic which is stable to penicillinase is purified by recrystn from isoPrOH and dried *in vacuo*. Its solubility in H_2O at 25° is 5%. [Doyle et al. *Nature* **192** 1183 *1961.*]

Oxolinic acid (5-ethyl-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-*g*]quinoline-3-carboxylic acid) *[14698-29-4]* **M 261.2, m 313-314°(dec), 314-316°(dec), pK_{Est} ~ 2.3.** Purified by recrystn from aqueous Me_2CO or 95% EtOH. It has UV λ_{max} 220, (255.5sh), 259.5, 268, (298sh, 311sh), 321 and 326nm [ε 14.8, (36.8sh), 38.4, 38.4, (6.4sh, 9.2sh), 10.8 and 11.2 x 10^3]. [Kaminsky and Mettzer *J Med Chem* **11** 160 *1968.*]

Oxytocin *[50-56-6]* **M 1007.2, m dec on heating, $[\alpha]_D^{22}$ -26.2° (c 0.53, N AcOH).** A cyclic nonapeptide which was purified by countercurrent distribution between solvent and buffer. It is soluble in H_2O, *n*-BuOH and isoBuOH. [Bodanszky and du Vigneaud *J Am Chem Soc* **81** 2504 *1959*; Cash et al. *J Med Pharm Chem* **5** 413 *1962*; Sakakibara et al. *Bull Chem Soc Jpn* **38** 120 *1965*; solid phase synthesis: Bayer and

Hagenmyer *Tetrahedron Lett* 2037 *1968*.] It was also synthesised on a solid phase matrix and finally purified as follows: A Sephadex G-25 column was equilibrated with the aqueous phase of a mixture of 3.5% AcOH (containing 1.5% of pyridine) + *n*-BuOH + $^{*}C_6H_6$ (2:1:1) and then the organic phase of this mixture was run through. A soln of oxytocin (100mg) in H_2O (2mL) was applied to the column which was then eluted with the organic layer of the above mixture. The fractions containing the major peak [as determined by the Folin-Lowry protein assay [Fryer et al. *Anal Biochem* **153** 262 *1986*] were pooled, diluted with twice their vol of H_2O, evaporated to a small vol and lyophilised to give oxytocin as a pure white powder (20mg, 508 U/mg). [Ives *Can J Chem* **46** 2318 *1968*.]

Palmitoyl coenzyme A *[1763-10-6]* **M 1005.9.**

Possible impurities are palmitic acid, S-palmitoyl thioglycolic acid and S-palmitoyl glutathione. These are removed by placing *ca* 200mg in a centrifuge tube and extracting with Me_2CO (20mL), followed by two successive extractions with Et_2O (15mL) to remove S-palmitoyl thioglycolic acid and palmitic acid. The residue is dissolved in H_2O (4 x 4 mL), adjusted to pH 5 and centrifuged to remove insoluble S-palmitoyl glutathione and other insoluble impurities. To the clear supernatant is added 5% $HClO_4$ (6mL) whereby S-palmitoyl CoA pptes. The ppte is washed with 0.8% $HClO_4$ (10mL) and finally with Me_2CO (3 x 5mL) and dried *in vacuo*. It is stable for at least one year in dry form at 0° in a desiccator (dark). Solns are stable for several months at -15°. Its solubility in H_2O is 4%. The adenine content is used as the basis of purity with λ_{max} at 260 and 232nm (ε 6.4 x 10^6 and 9.4 x 10^6 cm^2/mol respectively). Higher absorption at 232nm would indicate other thio ester impurities, e.g. S-palmitoyl glutathione, which absorb highly at this wavelength. Also PO_4 content should be determined and acid phosphate can be titrated potentiometrically. [Seubert *Biochem Prep* **7** 80 *1960*; Srer et al. *Biochim Biophys Acta* **33** 31 *1959*; Kornberg and Pricer *J Biol Chem* **204** 329 , 345 *1953*.]

3-Palmitoyl-sn-glycerol (*R*-glycerol-1-palmitate, L-β-palmitin) *[32899-41-5]* **M 330.5, $d^{27.3}$ 0.9014, m 66.5° (α-form), 74° (β'-form) and 77° (β-form).** The stable β-form is obtained by crystn from EtOH or Skellysolve B and recrystn from Et_2O provides the β'-form. The α-form is obtained on cooling the melt. [Malkin and el Sharbagy *J Chem Soc* 1631 *1936*; Chapman *J Chem Soc* 58 *1956*; Luton and Jackson *J Am Chem Soc* **70** 2446 *1948* .]

Pancuronium bromide (2β,16β–dipiperidino-5α-androstan-3α,17β–diol diacetate dimethobromide) *[15500-66-0]* **M 732.7, m 212-215°, 215°.** Odourless crystals with a bitter taste which are purified through acid-washed Al_2O_3 and eluted with isoPrOH-EtOAc (3:1) to remove impurities (e.g. the monomethobromide) and eluted with isoPrOH to give the pure bromide which can be recrystd from CH_2Cl_2-Me_2CO or isoPrOH-Me_2CO. It is soluble in H_2O (50%) and $CHCl_3$ (3.3%) at 20°. It is a non-depolarising muscle relaxant. [Buckett et al. *J Med Chem* **16** 1116 *1973*.]

D-Panthenol (Provitamin B, *R*-2,4-dihydroxy-3,3-dimethylbutyric acid 3-hydroxypropylamide) *[81-13-0]* **M 205.3, b 118-120°/0.02mm, d_{20}^{20} 1.2, n_D^{20} 1.4935, $[\alpha]_D^{20}$ (c 5, H_2O).** Purified by distn *in vacuo*. It is a slightly *hygroscopic* viscous oil. Soluble in H_2O and organic solvent. It is hydrolysed by alkali and strong acid. [Rabin *J Am Pharm Assoc (Sci Ed)* **37** 502 *1948*; Bonati and Pitré *Farmaco Ed Scient* **14** 43 *1959*.]

***R*-(+)-Pantothenic acid sodium salt (*N*-[2,4-dihydroxy-3,3-dimethylbutyryl] β-alanine Na salt)** *[867-81-2]* **M 241.2, $[\alpha]_D^{25}$ +27.1° (c 2, H_2O), pK^{25} 4.4 (for free acid).** Crystd from EtOH, very hygroscopic (kept in sealed ampoules). The free acid is a viscous hygroscopic oil with $[\alpha]_D^{25}$ +37.5° (c 5, H_2O), easily destroyed by acids and bases.

***R*-(+)-Pantothenic acid Ca salt** *[(D(+)- 137-08-6; 63409-48-3]* **M 476.5, m 195-196°, 200-201°, $[\alpha]_D^{20}$ +28.2° (c 5, H_2O).** Crysts in needles from MeOH, EtOH or isoPrOH (with 0.5mol of isoPrOH). Moderately *hygroscopic*. The *S-benzylisothiuronium salt* has **m** 151-152° (149° when crystd from Me_2CO). [Kagan et al. *J Am Chem Soc* **79** 3545 *1957*; Wilson et al. *J Am Chem Soc* **76** 5177 *1954*; Stiller and Wiley *J Am Chem Soc* **63** 1239 *1941*.]

Next Page