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L-Canavanine sulfate (from jackbean, *O*-guanidino-L-homoserine) *[2219-31-0]* **M 274.3, m 160-165°(dec), 172°(dec), $[\alpha]_D^{18.5}$ +19.8° (c 7, H_2O), pK_1^{25} 7.40 (CO_2H), pK_2^{25} 9.25 (α-NH_2), pK_3^{25} 11.5 (guanidinoxy).** Recrystd by dissolving (~1g) in H_2O (10mL), and adding with stirring 0.5 to 1.0 vols of 95% EtOH whereby crystals separate. These are collected, washed with Me_2CO-EtOH (1:1) and dried over P_2O_5 in a vacuum. [Hunt and Thompson *Biochem Prep* **13** 416 *1971*; Feacon and Bell *Biochem J* **59** 221 *1955*.]

Carbonic anhydrase (carbinate hydrolase) *[9001-03-0]* **M_r 31,000 [EC 4.2.1.1].** Purified by hydroxylapatite and DEAE-cellulose chromatography [Tiselius et al. *Arch Biochem Biophys* **65** 132 *1956*, *Biochim Biophys Acta* **39** 218 *1960*], and is then dialysed for crystn. A 0.5 to 1% soln of the enzyme in 0.05 M Tris-HCl pH 8.5 was dialysed against 1.75M soln of $(NH_4)_2SO_4$ in the same buffer, and this salt soln was slowly increased in salt concn by periodic removal of small amounts of dialysate and replacement with an equal volume of 3.5M $(NH_4)_2SO_4$. The final salt concn in which the DEAE-cellulose fractions which gave beautiful birefringent suspensions of crystals ranged from 2.4 to 2.7M, and appeared first as fine crystals then underwent transition to thin fragile plates. Carbonic anhydrase is a Zn enzyme which exists as several isoenzymes of varying degrees of activity [*J Biol Chem* **243** 6474 *1968*; crystal structure: *Nature, New Biology* 235 131 *1972*; see also P.D. Boyer Ed. *The Enzymes* Academic Press NY, pp 587-665 *1971*].

Carboxypeptidase A (from bovine pancreas, peptidyl-L-aminoacid lyase) *[11075-17-5]* **M_r 34,600 [EC 3.4.17.1]**. Purified by DEAE-cellulose chromatography, activation with trypsin and dialysed against 0.1M NaCl, yielding crystals. It is recrystd by dissolving in 20 mL of M NaCl and dialysed for 24hours each against the following salts present in 500mL of 0.02M sodium veronal pH 8.0: ,0.5M NaCl, 0.2M NaCl and 0.15M NaCl. The last dialysate usually induces crystn. If it does not crystallise then dialyse the last soln against 0.02M sodium veronal containing 0.10M NaCl. Only 2 or 3 recrystns are required to attain maximum activity. [Cox et al. *Biochemistry* **3** 44 *1964*.] Enzyme activity is measured by hydrolysing hippuryl-L-phenylalanine (or phenylacetic acid) and observing the rate of change of optical density at 254nm (reaction extinction coefficient is ~0.592 cm^2/µmole at pH 7.5 [Bergmyer *Methods in Enzymatic Analysis* (Academic Press) **1** 436 *1974*].

Carminic acid (7-α-D-glucopyranosyl-9,10-dihydro-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-2-anthracene carboxylic acid, Neutral Red 4: CI 75470) *[1260-17-9]* **M 492.4, m 120°(dec), $[\alpha]_{654}^{15}$ +51.6° (H_2O), (several phenolic pKs).** Forms red prisms from EtOH. It gives a red colour in Ac_2O and yellow to violet in acidic solution. UV: λ_{max} (H_2O) 500nm (ε 6,800); (0.02N HCl) 490-500nm (ε 5,800) and (0.0001N NaOH) 540nm (ε 3,450). IR: ν_{max} (Nujol) 1708s, 1693s, 1677m,1648m, 1632m, 1606s, 1566s, 1509 cm^{-1}. Periodate oxidation is complete after 4h at 0° with the consumption of 6.2 mols. The *tetra-O-methyl carminate* has **m** 186-188° (yellow needles from $^*C_6H_6$ + pet ether). [IR: Ali and Haynes *J Chem Soc* 1033 *1959*; Bhatia and Venkataraman *Indian J Chem* **3** (2) 92 *1965*; Synthesis: Davis and Smith *Biochem Prep* **4** 38 *1955*.]

Carnitine (α-hydroxy-β-*N,N,N*-trimethylaminopropionic acid) *[R(+)- 541-14-0 ; S(L-) 541-15-1; RS 461-06-3]* **M 161.2, m *R* or *S isomer* 197-198°(dec), 210-212°(dec), *RS isomer* 195-197°, $[\alpha]_{546}^{20}$ (+) and (-) 36° (c 10, H_2O), pK^{25} 3.6.** The *S*(L) isomer is **levocarnitine, Vitamin B_7**. The *R* or *S* isomers crystallise from EtOH + Me_2CO (hygroscopic). The *R* or *S hydrochlorides* crystallise from hot EtOH and have **m** 142°(dec). The *RS* isomer crystallises from hot EtOH (hygroscopic). The *RS hydrochloride* crystallises in needles from hot EtOH and has **m** 196°(dec).

L-Carnosine (β-alanyl-L-histidine) *[305-84-0]* **M 226.2, m 258-260°(dec), 260°(capillary tube), 262°(dec), $[\alpha]_D^{25}$ +20.5° (c 1.5, H_2O), pK_1^{25} 2.64, pK_2^{25} 6.83, pK_3^{25} 9.51.** Likely impurities: histidine, β-alanine. Crystd from water by adding EtOH in excess. Recrystd from aqueous EtOH by slow addition of EtOH to a strong aqueous soln of the dipeptide. Its solubility in H_2O is 33.3% at 25°. [Vinick and Jung *J Org Chem* **48** 392 *1983*; Turner *J Am Chem Soc* **75** 2388 *1953*; Sifford and du Vigneaud *J Biol Chem* **108** 753 *1935*.]

α-Carotene *[7488-99-5]* **M 536.9, m 184-188°, $[\alpha]^{20}_{643}$ +385° (c 0.08, *C_6H_6) λ_{max} 422, 446, 474 nm, in hexane, $A^{1\%}_{1cm}$ 2725 (at 446nm), 2490 (at 474nm).** Purified by chromatography on columns of calcium hydroxide, alumina or magnesia. Crystd from CS_2/MeOH, toluene/MeOH, diethyl ether/pet ether, or acetone/pet ether. Stored in the dark, under inert atmosphere at -20°.

***all-trans*-β-Carotene** *[7235-40-7]* **M 536.9, m 178-179°, 179-180°, 180°, 181°, 183° (evac capillary), $\varepsilon^{1\%}_{1cm}$ 2590 (450nm), 2280 (478nm), in hexane.** It forms purple prisms when crystd from *C_6H_6-MeOH and red rhombs from pet ether. Its solubility in hexane is 0.1% at 0°. It is **oxygen sensitive** and should be stored under N_2 at -20° in the dark. It gives a deep blue colour with $SbCl_3$ in $CHCl_3$. UV: (*C_6H_6) 429infl, λ_{max} 454 and 484nm. The principal peak at 454nm has $A^{1\%}_{1cm}$ 2000. [Synthesis: Surmatis and Ofner *J Org Chem* **26** 1171 *1961*; Milas et al. *J Am Chem Soc* **72** 4844 *1950*.] β-Carotene was also purified by chromatography (Al_2O_3 activity I-II) - it was dissolved in pet ether-*C_6H_6 (10:1), applied to the column and eluted with pet ether-EtOH, the desired fraction was evaporated and the residue recrystd from *C_6H_6-MeOH as violet-red plates. [UV: Inhoffen et al. *Justus Liebigs Ann Chem* **570** 54,68 *1950*; Review: Fleming *Selected Organic Synthesis* (J Wiley, Lond) pp. 70-74 *1973*.] Alternatively it can be purified by chromatography on a magnesia column, thin layer of Kieselguhr or magnesia. Crystd from CS_2/MeOH, Et_2O/pet ether, acetone/pet ether or toluene/MeOH. Stored in the dark, under inert atmosphere, at -20°. Recrystd from 1:1 EtOH/$CHCl_3$ [Bobrowski and Das *J Phys Chem* **89** 5079 *1985*; Johnston and Scaiano *J Am Chem Soc* **108** 2349 *1986*].

γ-Carotene *[472-93-5, 10593-83-6]* **M 536.9, $A^{1\%}_{1cm}$ (λmax) 2055 (437nm), 3100 (462nm), 2720 (494nm) in hexane.** Purified by chromatography on alumina or magnesia columns. Crystd from *C_6H_6/MeOH (2:1). Stored in the dark, under inert atmosphere, at 0°.

ξ-Carotene *[38894-81-4]* **M 536.9, m 38-42°, λ_{max} 378, 400, 425nm, $A^{1\%}_{1cm}$ (λmax) 2270 (400nm), in pet ether.** Purified by chromatography on 50% magnesia-HyfloSupercel, developing with hexane and eluting with 10% EtOH in hexane. It was crystd from toluene/MeOH. [Gorman et al. *J Am Chem Soc* **107** 4404 *1985*.] Stored in the dark under inert atmosphere at -20°.

λ–Carrageenan *[9064-57-7, 9000-07-1 (κ + little of λ)]*. This D-galactose-anhydro-D or L-galactoside polysaccharide is ppted from 4g of Carrageenan in 600mL of water containing 12g of KOAc by addn of EtOH. The fraction taken, ppted between 30 and 45% (v/v) EtOH. [Pal and Schubert *J Am Chem Soc* **84** 4384 *1962*.]

Cation exchange resins. Should be conditioned before use by successive washing with water, EtOH and water, and taken through two H^+-OH^--H^+ cycles by successive treatment with M HCl, water, M NaOH, water and M HCl, then washed with water until neutral to give the H^+ form. (See commercial catalogues on ion exchange resins).

Cathepsin B (from human liver) *[9047-22-7]* **M_r 27,500 [EC 3.4.22.1].** Purified by affinity chromatography on the semicarbazone of Gly-Phe-glycinal-linked to Sepharose 4B, with elution by 2,2'-dipyridyl disulfide [Rich et al. *Biochem J* **235** 731 *1986*; *Methods Enzymol* **80** 551 *1981*].

Cathepsin D (from bovine spleen) *[9025-26-7]* **M_r 56,000, [EC 3.4.23.5].** Purified on a CM column after ammonium sulfate fractionation and dialysis, then starch-gel electrophoresis and by ultracentrifugal analysis. Finally chromatographed on a DEAE column [Press et al. *Biochem J* **74** 501 *1960*].

Cephalosporin C potassium salt *[28240-09-7]* **M 453.5, $[\alpha]^{20}_D$ +103° (H_2O), pK_1 <2.6, pK_2 3.1, pK_3 9.8.** Purified by dissolving in the minimum volume of H_2O (filter) and adding EtOH until separation of solid is complete. A soln is stable in the pH range 2.5-8. It has UV λ_{max} is 260nm (log ε 3.95) in H_2O. The Ba salt has $[\alpha]^{20}_D$ +80° (c 0.57, H_2O) [Woodward et al. *J Am Chem Soc* **88** 852 *1966;* Abraham and Newton *Biochem J* **79** 377 *1961*; Hodgkin and Maslen *Biochem J* **79** 402 *1961*; see also *Quart Reviews Chem Soc* London **21** 231 *1967*].

Ceruloplasmin (from human blood plasma) *[9031-37-2]* **M_r 134,000.** This principle Cu transporter (90-90% of circulating Cu) is purified by precipitation with polyethylene glycol 4000, batchwise adsorption and elution from QAE-Sephadex, and gradient elution from DEAE-Sepharose CL-6B. Ceruloplasmin

was purified 1640-fold. Homogeneous on anionic polyacrylamide gel electrophoresis (PAGE), SDS-PAGE, isoelectric focusing and low speed equilibrium centrifugation. [Oestnuizen *Anal Biochem* **146** 1 *1985*; Cohn et al. *J Am Chem Soc* **68** 459 *1946*.]

Chemokines. These are small proteins formed from longer precursors and are chemoattractants for lymphocytes and lymphoid organs. They are characterised by having cysteine groups in specific relative positions. The two largest families are the α and β families that have four cysteine residues arranged (C-X-C) and (C-C) respectively. The mature chemokines have ~70 amino acids with internal cys S-S bonds and attract myeloid type cells *in vitro*. The γ-family (Lymphotactin) has only two cys residues. The δ-family (Neurotactin, Fractalkine) has the C-C-X-X-X-C sequence (*ca* 387 amino acids), binds to membrane promoting adhesion of lymphocytes. The soluble domain of human Fractalkine chemoattracts monocytes and T cells. Several chemokines are available commercially (some prepared by recombinant DNA techniques) including 6Ckine/exodus/SLC which belongs to the β-family with 6 cysteines (110 amino acids mature protein), as the name implies (C-C-C-C-X.....X-C-C) and homes lymphocytes to secondary lymphoid organs with lymphocyte adhesion antitumor properties. Other chemokines available are C10 (βCC) and Biotaxin. Several chemokine receptors and antibodies are available commercially and can generally be used without further purification. [Murphy 'Molecular biology of lymphocyte chemoattractant receptors' in *Ann Rev Immunol* **12** 593 *1994*.]

Chirazymes. These are commercially available enzymes e.g. lipases, esterases, that can be used for the preparation of a variety of optically active carboxylic acids, alcohols and amines. They can cause regio and stereospecific hydrolysis and do not require cofactors. Some can be used also for esterification or trans-esterification in neat organic solvents. The proteases, amidases and oxidases are obtained from bacteria or fungi, whereas esterases are from pig liver and thermophilic bacteria. For preparative work the enzymes are covalently bound to a carrier and do not therefore contaminate the reaction products. Chirazymes are available form Roche Molecular Biochemicals and are used without further purification.

Chlorambucil [4-{bis(2-chloroethyl)amino}benzene)butyric acid] *[305-03-3]* **M 304.2, m 64-66°, pK$_1$ 5.8 (6.0 at 66°, 50% aq Me_2CO), pK$_2$ 8.0.** It is recrystd from pet ether (flat needles) and has a solubility at 20° of 66% in EtOH, 40% in $CHCl_3$, 50% in Me_2CO but is insoluble in H_2O [Everett et al. *J Chem Soc* 2386 *1953*]. **CARCINOGEN.**

Chloramphenicol [Amphicol, 1*R*,2*R*-(-)-2-{2,2-dichloroacetylamino}-1-{4-nitrophenyl}-propan-1,3-diol] *[56-75-7]* **M 323.1, m 149-151°, 150-151°, 151-152°, $[\alpha]_D^{20}$ +20.5° (c 3, EtOH), $[\alpha]_D^{25}$ -25.5° (EtOAc).** Purified by recrystn from H_2O (sol 2.5mg/mL at 25°) or ethylene dichloride as needles or long plates and by sublimation at high vacuum. It has $A_{1cm}^{1\%}$ 298 at λ_{max}278nm and it is slightly soluble in H_2O (0.25%) and propylene glycol (1.50%) at 25° but is freely soluble in MeOH, EtOH, BuOH, EtOAc and Me_2CO. [Relstock et al. *J Am Chem Soc* **71** 2458 *1949*; Confroulis et al. *J Am Chem Soc* **71** 2463 *1949*; Long and Troutman *J Am Chem Soc* **71** 2469, 2473 *1949*, Ehrhart et al. *Chem Ber* **90** 2088 *1957*.]

Chloramphenicol palmitate *[530-43-8]* **M 561.5, m 90°, $[\alpha]_D^{26}$ +24.6° (c 5, EtOH).** Crystd from *benzene.

2-Chloroadenosine *[146-77-0]* **M 301.7, m 145-146°(dec), 147-149°(dec), pK$_{Est(1)}$~ 0.5, pK$_{Est(2)}$~ 7.6.** Purified by recrystn from H_2O (~1% in cold) and has λ_{max} at 264 nm (pH 1 and 7) and 265 nm (pH 13) in H_2O. [Brown and Weliky *J Org Chem* **23** 125 *1958*; Schaeffer and Thomas *J Am Chem Soc* **80** 3738 *1958*; IR: Davoll and Lewy *J Am Chem Soc* **74** 1563 *1952*.]

Chlorophylls *a* and *b* see entries on p. 167 in Chapter 4.

6-Chloropurine riboside (6-chloro-9-β-D-ribofuranosyl-9*H*-purine) *[2004-06-0]* **M 286.7, m 158-162°(dec), 165-166°(sintering At 155°), 168-170°(dec), $[\alpha]_D^{26}$ -45° (c 0.8, H_2O).** Purified by suspending the dry solid (~12 g) in hot MeOH (130 mL) and then adding enough hot H_2O (~560 mL) to cause solution, filter and set aside at 5° overnight. The colourless crystals of the riboside are filtered off, washed with Me_2CO, Et_2O and dried at 60°/0.1mm. More material can be obtained from the filtrate by evapn to

dryness and recrystn of the residue from MeOH-H_2O (2:1) (15mL/g). It has λ_{max} 264nm (ε 9140) in H_2O. [Robins *Biochem Prep* **10** 145 *1963*; Baker et al. *J Org Chem* **22** 954 *1957*.]

Chromomycin A$_3$ *[7059-24-7]* **M 1183.3, m 185°dec, $[\alpha]_D^{23}$ -57° (c 1, EtOH).** Dissolve reagent (10g) in EtOAc and add to a column of Silica Gel (Merck 0.05-0.2microns, 4x70cm) in EtOAc containing 1% oxalic acid. Elute with EtOAc+1% oxalic acid and check fractions by TLC. Pool fractions, wash with H_2O thoroughly, dry and evaporate. Recryst from EtOAc. The *hepta-acetate* has **m** 214°, $[\alpha]_D^{23}$ -20° (c 1, EtOH). [*Tetrahedron* **23** 421 *1967*; *J Am Chem Soc* **91** 5896 *1969*.]

α-Chymotrypsin *[9004-07-3]* **M_r ~25000 [EC 3.4.21.1].** Crystd twice from four-tenths saturated ammonium sulfate soln, then dissolved in 1mM HCl and dialysed against 1mM HCl at 2-4°. The soln was stored at 2° [Lang, Frieden and Grunwald *J Am Chem Soc* **80** 4923 *1958*].

Cinchonidine *[485-71-2]* **M 294.4, m 210.5°, $[\alpha]_{546}^{20}$ -127.5° (c 0.5, EtOH), pK_1^{15} 4.17, pK_2^{15} 8.4.** Crystd from aqueous EtOH. For *N-benzyl chloride* see entry in Chapter 6.

Cinchonine *[118-10-5]* **M 294.4, m 265°, $[\alpha]_{546}^{20}$ +268° (c 0.5, EtOH), pK_1^{15} 4.28, pK_2^{15} 8.35.** Crystd from EtOH or diethyl ether. For *N-benzyl chloride* see entry in Chapter 6.

Citranaxanthin *[3604-90-8]* **M 456.7, m 155-156°, $A_{1cm}^{1\%}$ (λmax) 410 (349nm), 275 (466nm) in hexane.** Purified by chromatography on a column of 1:1 magnesium oxide and HyfloSupercel (diatomaceous filter aid). Crystd from pet ether. Stored in the dark, under inert atmosphere, at 0°.

Citric acid cycle components (from rat heart mitochondria). Resolved by anion-exchange chromatography [LaNoue et al. *J Biol Chem* **245** 102 *1970*].

Clonidine hydrochloride [Catapres, 2-(2,6-dichloroanilino)-2-imidazoline hydrochloride] *[4205-91-8]* **M 266.6, m 305°, pK 5.88 (free base).** It is recrystd from EtOH-Et_2O and dried in a vacuum (solubility in H_2O is 5%). It has a pKa of 5.88. The *free base* has **m** 124-125° and is recrystallised from hexane. [Jen et al. *J Med Chem* **18** 90 *1975*; NMR: Jackman and Jen *J Am Chem Soc* **97** 2811 *1975*.]

Clostripain *[9028-00-6]* **[EC 3.4.22.8] M_r ~55,000.** Isolated from *Clostridium histolyticum* callogenase by extraction in pH 6.7 buffer, followed by hydroxylapatite chromatography with a 0.1-0.2 M phosphate gradient, then Sephadex G-75 gel filtration with 0.05M phosphate pH 6.7, dialysis and a second hydroxylapatite chromatography (gradient elution with 0.1M → 0.3M phosphate, pH 6.7). It has proteinase and esterase activity and is assayed by hydrolysing *n*-benzoyl-L-arginine methyl ester. [Mitchell and Harrington *J Biol Chem* **243** 4683 *1968*, *Methods Enzymol* **19** 635 *1970*.]

Cloxacillin sodium salt (sodium 3-*o*-chlorophenyl-5-methyl-4-isoxazolyl penicillin monohydrate) *[642-78-4]* **M 457.9, m 170°, $[\alpha]_D^{20}$ +163° (H_2O pH 6.0-7.5), pK_{Est} ~ 2.8 (COOH).** Purified by dissolving in isoPrOH containing 20% of H_2O, and diluting with isoPrOH to a water content of 5% and chilled, and recrystd again in this manner. The sodium salt is collected and dried at 40° in air to give the colourless monohydrate. It is soluble in H_2O (5%), MeOH, EtOH, pyridine and ethylene glycol. [Doyle et al. *J Chem Soc* 5838 *1963*; Naylor et al. *Nature* **195** 1264 *1962*.]

β-Cocaine {2β-carbomethoxy-3-β-benzoxytropane, methyl [1*R*-(*exo,exo*)]-3-(benzoyloxy)-2-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate} *[50-36-2]* **M 303.4, m 98°, b 187-188°/0.1mm, $[\alpha]_D^{20}$ -15.8° (c 4, $CHCl_3$), pK^{24} 8.39.** Crystallises from EtOH and sublimes below 90° in a vacuum in a non-crystalline form.

Cocarboxylase tetrahydrate (aneurine pyrophosphoric acid tetrahydrate, thiamine pyrophosphoric acid tetrahydrate) *[136-09-4]* **M 496.4, m 220-222°(sinters at 130-140°), 213-214°, $pK_{Est(1)}$~2, $pK_{Est(2)}$~6, $pK_{Est(3)}$~9.** Recrystd from aqueous Me_2CO. [Wenz et al. *Justus Liebigs Ann Chem* **618** 210 *1958*; UV: Melnick *J Biol Chem* **131** 615 *1939*; X-ray: Carlisle and Cook *Acta Cryst (B)* **25** 1359 *1969*.] The *hydrochloride salt* has **m** 242-244°(dec), 241-243°(dec) or 239-240°(dec) and is

recrystd from aqueous HCl + EtOH, EtOH containing HCl or HCl + Me_2CO. [Weijlard *J Am Chem Soc* **63** 1160 *1941*; Synthesis: Weijlard and Tauber *J Am Chem Soc* **60** 2263 *1938*.]

Codeine *[76-57-3]* **M 299.4, m 154-156°, $[\alpha]_D^{20}$ -138° (in EtOH), pK^{25} 8.21.** Crystd from water or aqueous EtOH. Dried at 80°.

Coenzyme A trihydrate *[85-61-0]* **M 821.6, pK_1 4.0 (adenine NH_2), pK_2 6.5 (PO_4H), pK_3 9.6 (SH).** White powder best stored in an inert atmosphere in the dark in sealed ampoules after drying *in vacuo* over P_2O_5 at 34°. It has UV: λ_{max} 259 nm (ε 16,800) in H_2O. [Buyske et al. *J Am Chem Soc* **76** 3575 *1954*.] It is sol in H_2O but insol in EtOH, Et_2O and M_2CO. It is readily oxidised in air and is best kept as the more stable *trilithium salt* [Moffat and Khorana *J Am Chem Soc* **83** 663 *1961*; see also Beinert et al. *J Biol Chem* **200** 384 *1953*; De Vries et al. *J Am Chem Soc* **72** 4838 *1950*; Gregory et al. *J Am Chem Soc* **74** 854 *1952* and Baddiley *Adv Enzymol* **16** 1 *1955*].

Coenzyme Q_0 (2,3-Dimethoxy-5-methyl-1,4-benzoquinone, 3,4-dimethoxy-2,5-toluquinone, fumigatin methyl ether) *[605-94-7]* **M 182.2, m 56-58°, 58-60°, 59°.** It crystallises in red needles from pet ether (b 40-60°) and can be sublimed in high vacuum with a bath temperature of 46-48° [Ashley, Anslow and Raistrick *J Chem Soc* 441 *1938*; UV in EtOH: Vischer *J Chem Soc* 815 *1953*; UV in cyclohexane: Morton et al. *Helv Chim Acta* **41** 2343 *1858*; Aghoramurthy et al. *Chem Ind (London)* 1327 *1954*].

Coenzyme Q_4 (Ubiquinone-4, 2,3-dimethoxy-5-methyl-6-[3,7,11,15-tetramethyl-hexadeca-2*t*,6*t*,10*t*,14-tetraenyl]-[1,4]benzoquinone *[4370-62-1]* **M 454.7, m 30°, 33-45°, $A^{1\%}_{1cm}$ (275nm) 185.** A red oil purified by TLC chromatography on SiO_2 and eluted with Et_2O-hexane. Purity can be checked by HPLC (silica column using 7% Et_2O-hexane). It has λ_{max} 270 nm (ε 14,800) in pet ether. [NMR and MS: Naruta *J Org Chem* **45** 4097 *1980;* cf Morton *Biochemical Spectroscopy* (Adam Hilger, London, 1975) p 491]. It has also been dissolved in MeOH/EtOH (1:1 v/v) and kept at 5° until crystals appear [Lester and Crane *Biochim Biophys Acta* **32** 497 *1958*].

Coenzyme Q_9 (Ubiquinone-9, 2,3-dimethoxy-5-methyl-6-[3,7,11,15,19,23,27,31,35-nonamethyl-hexatriaconta-2*t*,6*t*,10*t*,14*t*,18*t*,22*t*,26*t*,30*t*,34-nonaenyl]-1,4-benzoquinone) *[303-97-9]* **M 795.3, m 40.5-42.5°, 44-45°, 45°.** Yellow crystals purified by recrystn from pet ether and by TLC chromatography on SiO_2 and eluted with Et_2O-hexane. Purity can be checked by HPLC (silica column using 7% Et_2O-hexane). It has λ_{max} 270nm (ε 14,850) in pet ether. [NMR and MS: Naruta *J Org Chem* **45** 4097 *1980;* Le et al. *Biochem Biophys Acta* **32** 497 *1958*; cf Morton *Biochemical Spectroscopy* (Adam Hilger, London, 1975) p 491; IR: Lester et al. *Biochim Biophys Acta* **33** 169 *1959*; UV: Rüegg et al. *Helv Chim Acta* **42** 2616 *1959*; Shunk *J Am Chem Soc* **81** 5000 *1959*.]

Coenzyme Q_{10} (Ubiquinone-10, 2,3-dimethoxy-5-methyl-6-[3,7,11,15,19,23,27,31,35,-39-decamethyl-tetraconta-2*t*,6*t*,10*t*,14*t*,18*t*,22*t*,26*t*,30*t*,34*t*,38-decaenyl]-1,4-benzoquinone) *[303-98-0]* **M 795.3, m 48-49°,49°, 49.5-50.5°, 50°.** Purified by recrystn from EtOH, EtOH + Me_2CO or Et_2O-EtOH and by chromatography on silica gel using isoPrOH-Et_2O (3:1) to give orange crystals. It has λ_{max} 270nm (ε 15,170) in pet ether. [Terao et al. *J Org Chem* **44** 868 *1979*; NMR and MS: Naruta et al. *J Org Chem* **45** 4097 *1980*; IR: Lester et al. *Biochem Biophys Acta* **42** 1278 *1959*, NMR: Planta et al. *Helv Chim Acta* **42** 1278 *1959*; cf Morton *Biochemical Spectroscopy* (Adam Hilger, London, 1975) p 491].

Colcemide (Demecocine) *[477-30-5]* **M 371.4, m 182-185°, 183-185°, 186°, $[\alpha]_D^{20}$ -129° (c 1, $CHCl_3$).** It has been purified by chromatography on silica and eluting with $CHCl_3$-MeOH (9:1) and recrystn from EtOAc-Et_2O and forms yellow prisms. UV in EtOH has λ_{max} 243nm (ε 30,200) and 350nm (ε 16,3000). [Synthesis, IR, NMR, MS: Capraro and Brossi *Helv Chim Acta* **62** 965 *1979*.]

Colchicine *[64-86-8]* **M 399.5, m 155-157°(dec), $[\alpha]_{546}^{20}$ -570° (c 1, H_2O), pK^{20} 1.85.** Commercial material contains up to 4% desmethylcolchicine. Purified by chromatography on alumina, eluting with $CHCl_3$ [Ashley and Harris *J Chem Soc* 677 *1944*]. Alternatively, an acetone solution on alkali-free alumina has been used, and eluting with acetone [Nicholls and Tarbell *J Am Chem Soc* **75** 1104 *1953*].

Colchicoside *[477-29-2]* **M 547.5, m 216-218°.** Crystd from EtOH.

Colicin E (from *E.coli*) *[11032-88-5].* Purified by salt extraction of extracellular-bound colicin followed by salt fractionation and ion-exchange chromatography on a DEAE-Sephadex column, and then by CM-Sephadex column chromatography [Schwartz and Helinski *J Biol Chem* **246** 6318 *1971*].

Collagenase (from human polymorphonuclear leukocytes) *[9001-12-1]* **M_r 68,000-125,000 [EC 3.4.24.3].** Purified by using *N*-ethylmaleimide to activate the enzyme, and wheat germ agglutinin-agarose affinity chromatography [Callaway et al. *Biochemistry* **25** 4757 *1986*].

Compactin *[73573-88-3]* **M 390.5, m 151-153°, 152°, $[\alpha]_D^{22}$ +283° (c 0.48, acetone).** Purified by recrystn from aqueous EtOH. UV: λ_{max} 230, 237 and 246nm (log ε 4.28, 4.30 and 4.11); IR (KBr): ν 3520, 1750 (lactone CO) and 1710 (CO ester) cm^{-1}. [Clive et al. *J Am Chem Soc* **110** 6914 *1988*; Synthesis review: Rosen and Heathcock *Tetrahedron* **42** 4909 *1986*; IR, NMR, MS: Brown et al. *J Chem Soc Perkin Trans 1* 1165 *1976*.]

Convallatoxin (α cardenolide mannoside) *[508-75-8]* **M 550.6, m 238-239°, $[\alpha]_D^{25}$ 9.4° (c 0.7, dioxane).** Crystd from EtOAc. *Tetra-acetate* has **m** 238-242° (MeOH/Et_2O), $[\alpha]_D^{25}$ -5° ($CHCl_3$).

Copper-zinc-superoxide dismutase (from blood cell haemolysis) *[9054-89-1]* **M_r ~32,000 [EC 1.15.1.1].** Purified by DEAE-Sepharose and copper chelate affinity chromatography. The preparation was homogeneous by SDS-PAGE, analytical gel filtration chromatography and by isoelectric focusing [Weselake et al. *Anal Biochem* **155** 193 *1986*; Fridovich *J Biol Chem* **244** 6049 *1969*].

Coproporphyrin I *[531-14-6]* **M 654.7, λ_{max} 591, 548, 401nm in 10% HCl.** Crystd from pyridine/glacial acetic acid. The *dihydrochloride [69477-27-6]* has M 727.7 and λmax 395nm in water.

Corticosterone (11β, 21-dihydroxypregn-4-en-3,20-dione) *[50-22-6]* **M 346.5, m 180-181°, 180-182°, 181-184°, $[\alpha]_D^{15}$ +223° (c 1.1, EtOH), $[\alpha]_D^{23-25}$ +194° (c 0.1, dioxane).** Purified by recrystn from Me_2CO (trigonal plates), EtOH or isoPrOH. UV λ_{max} at 240nm, and gives an orange-yellow soln with strong fluorescence on treatment with concentrated H_2SO_4. Insoluble in H_2O but soluble in organic solvents. [Reichstein and Euw *Helv Chim Acta* **21** 1197 *1938*, **27** 1287 *1944*; Mason et al. *J Biol Chem* **114** 613 *1936*; ORD: Foltz et al. *J Am Chem Soc* **77** 4359 *1955*; NMR: Shoolery and Rogers *J Am Chem Soc* **80** 5121 *1958*.] The *21-O-benzoyl* derivative has **m** 201-202° [Reichstein *Helv Chim Acta* **20** 953 *1937*].

Corticotropin *[92307-52-3]* **polypeptide M_r ~4697.** Extract separated by ion-exchange on CM-cellulose, desalted, evapd and lyophilised. Then run on gel filtration (Sephadex G-50) [Lande et al. *Biochemical Preparations* **13** 45 *1971*; Esch et al. *Biochem Biophys Res Commun* **122** 899 *1984*].

Cortisol see hydrocortisone on p. 541.

Cortisone *[53-06-5]* **M 360.5, m 230-231°, $[\alpha]_{546}^{20}$ +225° (c 1, in EtOH).** Crystd from 95% EtOH or acetone.

Cortisone-21-acetate *[50-04-4]* **M 402.5, m 242-243°, $[\alpha]_{546}^{20}$ +227° (c 1, in $CHCl_3$).** Crystd from acetone.

Creatine (H_2O) (*N*-guanidino-*N*-methylglycine) *[6020-87-7]* **M 131.1, m 303°, pK_1^{25} 2.63, pK_2^{25} 14.3.** Likely impurities are creatinine and other guanidino compounds. Crystd from water as monohydrate. Dried under vacuum over P_2O_5 to give anhydrous material.

Creatine phosphate di Na, $4H_2O$ salt (phosphocreatine) *[922-32-7]* **M 327.1, pK_1^{27} 2.7, pK_2^{27} 4.58, pK_3^{27}~ 12.** To 3-4g of salt in H_2O (220mL) is added 4 vols of EtOH with thorough stirring and allowed to stand at 20° for 12hrs (this temp is critical as crystals did not readily form at 23° or 25°). The salt first appears as oily droplets which slowly settle and crystallise. After 12hrs the supernatant is clear. Stirring

and scratching the flask containing the filtrate brings out additional (0.3-1g) crystals if the salt is kept at 20° for 12hrs. Filter at room temp, wash with 3 x 5mL of ice-cold 90% EtOH then 5mL of abs EtOH and dry in a vac desiccator (Drierite or $CaCl_2$) for 16-30hrs. The hexahydrate (plates) is converted to the tetrahydrate salt (needles) in vac at -10°. [Ennor and Stocken *Biochem Prep* **5** 9 *1957*; *Biochem J* **43** 190 *1958*.]

Creatinine (2-imino-1-methyl-4-oxoimidazolidine) *[60-27-5]* **M 113.1, m 260°(dec), pK_1^{25} 4.80, pK_2^{25} 9.2.** Likely impurities are creatine and ammonium chloride. Dissolved in dilute HCl, then neutralised by adding ammonia. Recrystd from water by adding excess of acetone.

Crotaline (monocrotaline, 12,13-dihydroxy-(13β-14β*H*)-14,19-dihydro-20-norcrotalanan-11,15-dione) *[315-22-0]* **M 325.4, m 196-197°(dec), 197-198°(dec), 203°(dec), $[\alpha]_D^{20}$ -55° (c 1, EtOH).** It forms prisms from absolute EtOH and recrystallises also from $CHCl_3$. UV in 96% EtOH has λ_{max} 217nm (log ε 3.32). [Adams et al. *J Am Chem Soc* **74** 5612 *1952*; Culvenor and Smith *Aust J Chem* **10** 474 *1957*.] The *hydrochloride* has **m** 212-214° (from MeOH-Et_2O) and $[\alpha]_D^{28}$ -38.4° (c 5, H_2O) [Adams and Gianturco *J Am Chem Soc* **78** 1922 *1956*]. The *picrate* has **m** 230-231.5°(dec) [Adams et al. *J Am Chem Soc* **74** 5614 *1952*].

α-Cyclodextrin (H_2O) *[10016-20-3]* **M 972.9, m >280°(dec), $[\alpha]_{546}$ +175° (c 10, H_2O).** Recrystd from 60% aq EtOH, then twice from water, and dried for 12hours in a vacuum at 80°. Also purified by pptn from water with 1,1,2-trichloroethylene. The ppte was isolated, washed and resuspended in water. This was boiled to steam distil the trichloroethylene. The soln was freeze-dried to recover the cyclodextrin. [Armstrong et al. *J Am Chem Soc* **108** 1418 *1986*].

ß-Cyclodextrin (H_2O) *[7585-39-9]* **M 1135.0, m >300°(dec), $[\alpha]_{546}$ +170° (c 10, H_2O).** Recrystd from water and dried for 12hours in a vacuum at 110°, or 24hours in a vacuum at 70°. The purity was assessed by TLC on cellulose with a fluorescent indicator. [Taguchi, *J Am Chem Soc* **108** 2705 *1986*; Tabushi et al. *J Am Chem Soc* **108** 4514 *1986*; Orstam and Ross *J Phys Chem* **91** 2739 *1987*.]

D-(*R-natural*) and L-(*S*-non-natural) Cycloserine (2-amino-3-isoxazolidone) *[R- 68-41-7 and S- 339-72-0]* **M 102.1, m 145-150° (dec), 154-155°, 155-156° (dec), 156° (dec), $[\alpha]_D^{25}$ (+) and (-) 137° (c 5, 2N NaOH), pK_1^{10} 4.5, pK_2^{10} 7.74, pK_1^{25} 4.50, pK_2^{25} 7.43, pK_1^{50} 4.44, pK_2^{50} 7.20.** Purified by recrystn from aqueous EtOH or MeOH or aqueous NH_3 + EtOH or isoPrOH. Also recrystd from aqueous ammoniacal soln at pH 10.5 (100mg/mL) by diluting with 5 volumes of isopropanol and then adjusting to pH 6 with acetic acid. An aqueous soln buffered to pH 10 with Na_2CO_3 can be stored in a refrigerator for 1 week without decomposition. UV: λ_{max} 226nm ($A_{1cm}^{1\%}$ 4.02). The *tartrate salt* has **m** 165-166° (dec), 166-168° (dec), and $[\alpha]_D^{24}$ -41° (c 0.7, H_2O). [Stammer et al. *J Am Chem Soc* **79** 3236 *1959*; UV: Kuehl *J Am Chem Soc* **77** 2344 *1955*.]

Cystamine dihydrochloride [2,2'-diaminodiethylene disulfide dihydrochloride, 2,3'-dithio-bis(ethylamine) dihydrochloride] *[56-17-7]* **M 225.2, m 219-220°(dec), pK_1^{30} 8.82, pK_2^{30} 9.58.** Recrystd by dissolving in EtOH containing a few drops of dry EtOH-HCl, filtering and adding dry Et_2O. The solid is dried in a vacuum and stored in dry and dark atmosphere. It has been recrystd from EtOH (solubility: 1g in 60mL of boiling EtOH) or MeOH (plates). The *free base* has **b** 90-100°/0.001mm, 106-108°/5mm and 135-136°/atm, d_4^{20} 1.1559, n_D^{20} 1.5720. [Verly and Koch *Biochem J* **58** 663 *1954*; Gonick et al. *J Am Chem Soc* **76** 4671 *1954*; Jackson and Block *J Biol Chem* **113** 137 *1936]*.] The *dihydrobromide* has **m** 238-239° (from EtOH-Et_2O) [Viscontini *Helv Chim Acta* **36** 835 *1953*].

***S,S*-(L,L)-Cystathionine (*S*-2-amino-2-carboxyethyl-L-homocysteine, L-2-amino-4[(2-amino-2-carboxyethyl)thio]butyric acid)** *[56-88-2]* **M 222.3, m >300°, dec at 312° with darkening at 270°, $[\alpha]_D^{20}$ +23.9° (c 1, M HCl).** Could be converted to the *HCl* salt by dissolving in 20% HCl and carefully basifying with aqueous NH_3 until separation is complete. Filter off and dry in a vacuum. It forms prisms from H_2O. The *dibenzoyl* derivative has **m** 229° (from EtOH). [IR: Greenstein and Winitz *Chemistry of the Amino Acids (J Wiley)* **Vol 3** 2690 *1961* and Tallan et al. *J Biol Chem* **230** 707 *1958*; Synthesis: du Vigneaud et al. *J Biol Chem* **143** 59 *1942*; Anslow et al. *J Biol Chem* **166** 39 *1946*.]

[Prepn: Weiss and Stekol *J Am Chem Soc* **73** 2497 *1951*; see also du Vigneaud et al. *J Biol Chem* **143** 60 *1942*; Biological synthesis: Greenberg *Methods Enzymol* **5** 943 *1962*.]

Cysteamine (2-aminoethanethiol, 2-mercaptoethylamine) *[60-23-1]* **M 77.2, m 97-98.5°, 98-99°, 99-100°, pK_1^0 9.15, pK_2^0 11.93, pK_1^{30} 8.42, pK_2^{30} 10.83.** Soluble in H_2O giving an alkaline reaction and it has a disagreeable odour. Likely impurity is the disulfide, cystamine which is not soluble in alkaline solution. Under a N_2 atmosphere dissolve in EtOH, evaporate to dryness and wash the white residue with dry pet ether, then sublime at 0.1mm and store under N_2 (out of contact with air) at 0-10° in the dark. Its *$HgCl_2$ (2:3) complex* has **m** 181-182° (from H_2O), and its *picrate* has **m** 125-126°. [Mills and Bogert *J Am Chem Soc* **57** 2328 *1935*, **62** 1173 *1940*; Baddiley and Thain *J Chem Soc* 800 1952; Shirley *Preparation of Organic Intermediates (J. Wiley)* **Vol 3** 189 *1951*; Barkowski and Hedberg *J Am Chem Soc* **109** 6989 *1987*.]

Cysteamine hydrochloride *[156-57-0]* **M 113.6, m 70.2-70.7°, 70-72°.** Purified by recrystn from EtOH. It is freely soluble in H_2O and should be stored in a dry atmosphere. [Mills and Bogert *J Am Chem Soc* **62** 1177 *1940*.] The *picrate* has **m** 125-126°, see previous entry for *free base*.

(±)-Cysteic acid (3-sulfoalanine, 1-amino-3-sulfopropionic acid) *[13100-82-8, 3024-83-7]* **M 169.2, m 260°(dec).** Likely impurities are cystine and oxides of cysteine. Crystd from water by adding 2 volumes of EtOH. When recrystd from aqueous MeOH it has **m** 264-266°, and the anhydrous acid has **m** ~260°(dec). [Chapeville and Formageot *Biochim Biophys Acta* **26** 538 *1957*; *J Biol Chem* **72** 435 *1927*.]

***R*(L)-Cysteic acid (H_2O)** *[23537-25-9]* **M 187.2, m 275-280° (dec), 289°, $[\alpha]_D^{20}$ +8.66° (c 7.4, H_2O, pH 1) and +1.54° (H_2O, pH 13), pK_1^{25} 1.9 (SO_3H), pK_2^{25} 8.7 (CO_2H), pK_3^{25} 12.7 (NH_2).** Likely impurities are cystine and oxides of cysteine. Crystd from water by adding 2 volumes of EtOH. When recrystd from aqueous MeOH it has **m** 264-266°, and the anhydrous acid has **m** ~260°(dec). [Chapeville and Formageot *Biochim Biophys Acta* **26** 538 *1957*; *J Biol Chem* **72** 435 *1927*.]

D-(*S*)- and L-(*R*)- Cysteine (*S*- and *R*-2-amino-3-mercaptopropionoic acid) *[S(+)-: 921-01-7* and *R(-)-: 52-90-4]* **M 121.2, m 230°, 240° (dec), $[\alpha]_D^{20}$ (+) and (-) 7.6° (c 2, M HCl) and (+) and (-) 10.1° (c 2, H_2O, pH 10), pK_1^{25} 1.92 (CO_2), pK_2^{25} 8.35 (NH_2), pK_3^{25} 10.46 (SH).** Purified by recrystn from H_2O (free from metal ions) and dried in a vacuum. It is soluble in H_2O, EtOH, Me_2CO, EtOAc, AcOH, $^*C_6H_6$ and CS_2. Acidic solns can be stored under N_2 for a few days without deterioration. [For synthesis and spectra see Greenstein and Winitz *Chemistry of the Amino Acids (J. Wiley)* **Vol 3** p1879 *1961*.]

L-Cysteine hydrochloride (H_2O) *[52-89-1]* **M 175.6, m 175-178° (dec), $[\alpha]_D^{25}$ +6.53° (5M HCl).** Likely impurities are cystine and tyrosine. Crystd from MeOH by adding diethyl ether, or from hot 20% HCl. Dried under vacuum over P_2O_5. *Hygroscopic.*

(±)-Cysteine hydrochloride *[10318-18-0]* **M 157.6.** Crystd from hot 20% HCl; dried under vacuum over P_2O_5.

L-Cystine *[56-89-3]* **M 240.3, $[\alpha]_D^{18.5}$ -229° (c 0.92 in M HCl), pK_1^{25} 1.04 (1.65), pK_2^{25} 2.05 (2.76), pK_3^{25} 8.00 (7.85), pK_4^{25} 10.25 (8.7, 9.85).** Cystine disulfoxide was removed by treating an aqueous suspension with H_2S. The cystine was filtered off, washed with distilled water and dried at 100° under vacuum over P_2O_5. Crystd by dissolving in 1.5M HCl, then adjusting to neutral pH with ammonia. Likely impurities are D-cystine, meso-cystine and tyrosine.

Cytidine *[65-46-3]* **M 243.2, m 210-220°(dec), 230° (dec), 251-252° (dec), $[\alpha]_{546}^{20}$ +37° (c 9, H_2O), $[\alpha]_D^{20}$+29° (c 9, H_2O), pK 3.85.** Crystd from 90% aqueous EtOH. Also has been converted to the *sulfate* by dissolving (~200mg) in a soln of EtOH (10mL) containing H_2SO_4 (50mg), whereby the salt crystallises out. It is collected, washed with EtOH and dried for 5hours at 120°/0.1mm. The *sulfate* has **m** 225°. The *free base* can be obtained by shaking with a weak ion-exchange resin, filtering, evaporating and recrystallising the residue from EtOH as before. [Fox and Goodman *J Am Chem Soc* **73** 3256 *1956*; Fox and

Shugar *Biochim Biophys Acta* **9** 369 *1952*; see Prytsas and Sorm in *Synthetic Procedures in Nucleic Acid Chemistry* (Zorbach and Tipson Eds) **Vol 1** 404 *1973*.]

Cytisine see entry in Chapter 4.

Cytochalasin B (from dehydrated mould matter) *[14930-96-2]* **M 479.6.** Purified by MeOH extraction, reverse phase C18 silica gel batch extraction, selective elution with 1:1 v/v hexane/tetrahydrofuran, crystn, subjected to TLC and recrystallised [Lipski et al. *Anal Biochem* **161** 332 *1987*].

Cytochrome c_1 (from horse, beef or fishes' heart, or pigeon breast muscle) *[9007-43-6]* **M ~ 13,000.** Purified by chromatography on CM-cellulose (CM-52 Whatman) [Brautigan et al. *Methods Enzymol* **53D** 131 *1978*]. It has a high PI (isoelectric point) and has been purified further by adsorption onto an acidic cation exchanger, e.g. Amberlite IRC-50 (polycarboxylic) or in ground form Amberlite XE-40 (100-200 mesh) or Decalso-F (aluminium silicate), where the non-cytochrome protein is not adsorbed and is readily removed. The cytochrome is eluted using a soln containing 0.25g ions/L of a univalent cation at pH 4.7 adsorbed onto the NH_4^+ salt of Amberlite IRC-50 at pH 7, washed with H_2O and then with 0.12M NH_4OAc to remove non-cytochrome protein. When the cytochrome begins to appear in the eluate then the NH_4OAc concn is increased to 0.25 M. The fractions with *ca* Fe = 0.465—0.467 are collected, dialysed against H_2O and adsorbed onto a small IRC-50 column and eluted with 0.5M NH_4OH, then dialysed and lyophilised. (A second fraction (II) can be eluted from the first resin with 0.5M NH_4OH but is discarded). [Keilin and Hartree *Biochem Prep* **1** 1 *1952*; Margoliash *Biochem Prep* **8** 33 *1957*.]
Cytochrome c has been recrystd as follows: The above eluate (*ca* 100mL) is dialysed against H_2O (10 vols) at 4° for 24 h (no more), then passed through an XE-40 column (2 x 1 cm above) which is equilibrated with 0.1M NH_4OAc pH 7.0. The column is washed with 0.1% $(NH_4)_2SO_4$ pH 8.0 and the dark red resin in the upper part of the column is collected and in 0.1% $(NH_4)_2SO_4$ pH 8.0 transferred to another column (7 mm diameter) and the cytochrome c is eluted with 5% $(NH_4)_2SO_4$ pH 8.0. More than 98% of the red colour is collected in a volume of *ca* 4mL in a weighed centrifuge tube. Add a drop of octanol, 0.43g of $(NH_4)_2SO_4$/g of soln. When the salt has dissolved ascorbic acid (5mg), add a few drops of 30% NH_3 and keep the soln at 10° for 10min (turns lighter colour due to reduction). Then add finely powdered $(NH_4)_2SO_4$ in small portions (stir with a glass rod) until the soln becomes turbid. Stopper the tube tightly, and set aside at 15-25° for 2 days while the cytochrome c separates as fine needles or rosettes. Further $(NH_4)_2SO_4$ (20mg) are added per mL of suspension and kept in the cold for a few days to complete the crystallisation. The crystals are collected by centrifugation (5000xg), suspended in saturated $(NH_4)_2SO_4$ (pH 8.0 at 10°) then centrifuged again. For recrystn the crystals are dissolved in the least volume of H_2O, one drop of ammonia and 1 mg of ascorbic acid are added and the above process is repeated. The yield of twice recrystd cytochrome c from 2Kg of muscle is *ca* 200 mg but this varies with the source and freshness of the muscle used. The crystals are stored as a solid after dialysis against 0.08M NaCl or 0.1M sodium buffer and lyophilising, or as a suspension in saturated $(NH_4)_2SO_4$ at 0°. [Hagihara et al. *Biochem Prep* **6** 1 *1958*.] *Purity of cytochrome c:* This is checked by the ratio of the absorbance at 500nm (reduced form) to 280nm (oxidised form), i.e. $\varepsilon_{500}/\varepsilon_{280}$ should be between 1.1 and 1.28, although values of up to 1.4 have been obtained for pure preparations.
For the preparation of the *reduced form* see Margoliash *Biochem Prep* **5** 33 *1957* and Yonetani *Biochem Prep* **11** 19 *1966*.
Cytochrome from *Rhodospirillum rubrum*. ($\varepsilon_{270}/\varepsilon_{551}$ 0.967). Purified by chromatography on a column of CM-Whatman cellulose [Paleus and Tuppy *Acta Chem Scand* **13** 641 *1959*].

Cytochrome c oxidase (from bovine heart mitochondria). *[9001-16-5]* **M_r 100,000/haeme, [EC 1.9.3.1].** Purified by selective solubilisation with Triton X-100 and subsequently with lauryl maltoside; finally by sucrose gradient centrifugation [Li et al. *Biochem J* **242** 417 *1978*].
Also purified by extraction in 0.02 M phosphate buffer (pH 7.4) containing 2% of cholic acid (an inhibitor which stabilises as well as solubilises the enzyme) and fractionated with $(NH_4)_2SO_4$ collecting the 26-33% saturation cut and refractionating again and collecting the 26-33% saturation fraction. The pellet collected at 10,000xg appears as an oily paste. The cholate needs to be removed to activate the enzyme as follows: The ppte is dissolved in 10mL of 0.1M phosphate buffer pH 7.4, containing 1% of Tween-80 and dialysed against 1L of 0.01 M PO_4 buffer (pH 7.4) containing 1% of Tween-80 for 10 h at 0° and aliquoted. The enzyme is

stable at 0° for 2 weeks and at -15° for several months. It is assayed for purity (see reference) by oxidation of reduced cytochrome c (Km 10μM). [Yonetani *Biochem Prep* **11** 14 *1966*; *J Biol Chem* **236** 1680 *1961*.]

Cytokines see chemokines, interferons, interleukins.

Cytosine *[71-30-7]* **M 111.1, m 320-325° (dec), pK_1^{25} 4.6, pK_2^{25} 12.1.** Crystd from water.

Cytosine-1-β-*O*-arabinofuranoside (Cytarabin) *[147-94-4]* **M 243.2, m ~220°(dec), 212-213.5°, $[\alpha]_D^{20}$ +155° (c 1, H_2O), pK^{25} 4.3.** Purified by recrystn from aqueous EtOH. It has λ_{max} 212 and 279nm at pH 2 and 272nm at pH 12. [Walwick et al. *Proc Chem Soc (London)* 84 *1959*.]

N-Decanoyl-*N*-methylglucamine

(Mega-10, *N*-D-glucidyl-*N*-methyl deconamide) *[85261-20-7]* **M 349.5, m 91-93°, 92°.** Possible impurities are decanoic acid and *N*-methylglycamine. The former is removed by grinding the solid with Et_2O and then with pet ether and dried over P_2O_5. Twice recrystd from MeOH-Et_2O by dissolving in the minimum volume of MeOH and adding Et_2O and drying in a vacuum. To remove the glycamine the solid (800mg) is dissolved in hot H_2O (10mL) and set aside. Mega-10 crystallises in colourless needles. These are filtered off and dried in a vacuum to constant weight. It is a good non-ionic non-hygroscopic detergent with a critical micelle concentration (CMC) of 7.4mM (0.26%) in 0.1M Tris-HCl pH 7.4 at 25°. [Hildreth *Biochem J* **207** 363 *1982*.]

Demeclocycline hydrochloride (7-chloro-6-demethyltetracycline hydrochloride, Clortetrin) *[64-73-3]* **M 501.3, m 174-178°(dec, for sesquihydrate), $[\alpha]_D^{25}$ -258° (c 0.5, 0.1N H_2SO_4), pK 4.45 [H_2O-Me_2NCHO (1:1)].** Crystd from EtOH-Et_2O or H_2O and dried in air [McCormick et al. *J Am Chem Soc* **79** 4561 *1957*; Dobrynin et al. *Tetrahedron Lett* 901 *1962*].

2'-Deoxyadenosine (adenine 2'-deoxyriboside) *[16373-93-6]* **M 269.3, m 187-189°, 189-191°, $[\alpha]_D^{20}$ -25° (c 0.5, H_2O), $[\alpha]_{589}^{25}$ -26°, $[\alpha]_{310}^{25}$ -206° (c 0.5, H_2O), pK^{20} 3.79.** Purified by recrystn from H_2O (as hydrated crystals; solubility of mono-hydrate is 1.1% in H_2O at 20°). It has λ_{max} 258nm (pH 1), 260nm (pH 7) and 261nm (pH 13). [Ness and Fletcher *J Am Chem Soc* **81** 4752 *1959*; Walker and Butler *Can J Chem* **34** 1168 *1956*.] The *3',5'-O-diacetyl derivative* has **m** 151-152° (recrystd from EtOAc-pet ether).

3'-Deoxyadenosine (Cordycepin, adenine 3'-deoxyriboside) *[73-03-0]* **M 251.2, m 225-226°, 225-229°, $[\alpha]_D^{20}$ -47° (H_2O), pK_{Est} ~ 4.8.** It forms needles from EtOH, *n*-BuOH and *n*-PrOH, and from H_2O as the mono-hydrate. It has λ_{max} 260nm (ε 14,600) in EtOH. The *picrate* has **m** 195°(dec, yellow crystals from H_2O). [Kaczka et al. *Biochim Biophys Acta* **14** 456 *1964*; Todd and Ulbricht *J Chem Soc* 3275 *1960*; Lee et al. *J Am Chem Soc* **83** 1906 *1961*; Walton et al. *J Am Chem Soc* **86** 2952 *1964*.]

11-Deoxycorticosterone acetate (21-acetoxy-4-pregnen-3,20-dione) *[56-47-3]* **M 372.5, m 154-159°, 154-160°, 155-157°, 155-161°, $[\alpha]_D^{20}$ +174° (c 1, dioxane), $[\alpha]_D^{22-24}$ +196° (c 1, $CHCl_3$).** Recrystallises from EtOH as needles or Me_2CO-hexane, and sublimes at high vacuum. Partly soluble in MeOH, Me_2CO, Et_2O and dioxane but insoluble in H_2O. [Romo et al. *J Am Chem Soc* **79** 5034 *1957*; NMR: Shoolery and Rogers *J Am Chem Soc* **80** 5121 *1959*.]

2'-Deoxycytidine monohydrate *[951-77-9]* **M 245.2, m 119-200°, 207-209°, 213-215°, $[\alpha]_D^{25}$ +78° (c 0.4, N NaOH), $[\alpha]_D^{23}$ +57.6° (c 2, H_2O), pK 4.25.** Purified by recrystn from MeOH-Et_2O or EtOH and dried in air. [NMR: Miles *J Am Chem Soc* **85** 1007 *1963*; UV: Fox and Shugar *Biochim Biophys Acta* **9** 369 *1952*.] The *hydrochloride* crystallises from H_2O-EtOH and has **m** 174°(dec, 169-173°) [Walker and Butler *Can J Chem* **34** 1168 *1956*.] The *picrate* has **m** 208°(dec). [Fox et al. *J Am Chem Soc* **83** 4066 *1961*.]

2'-Deoxycytidine 5'-monophosphoric acid (deoxycytidylic acid) *[1032-65-1]* **M 307.2, m 170-172°(dec), 183-184°(dec), 183-187°(dec), $[\alpha]_D^{21}$ +35° (c 0.2, H_2O), pK_1 4.6, pK_2 6.6.**

Recrystd from H_2O or aqueous EtOH and dried in a vacuum. [Volkin et al. *J Am Chem Soc* **73** 1533 *1951*; UV: Fox et al. *J Am Chem Soc* **75** 4315 *1953*; IR: Michelson and Todd *J Chem Soc* 3438 *1954*.]

2'-Deoxyguanosine monohydrate (9-[2-deoxy-β-D-ribofuranosyl]guanidine) *[961-07-9]* **M 285.3, m *ca* 200°(dec), $[\alpha]_D^{20}$ +37.5° (c 2, H_2O), $[\alpha]_D^{14}$ -47.7° (c 0.9, N NaOH), $pK_{Est(1)}$~ 3.3, $pK_{Est(2)}$~ 9.2.** Recrystd from H_2O as the monohydrate. [Brown and Lythgoe *J Chem Soc* 1990 *1950*; Levene and London *J Biol Chem* **81** 711 *1929*, **83** 793 *1929*]; UV: Hotchkiss *J Biol Chem* **175** 315 *1948*; ORD: Levendahl and James *Biochim Biophys Acta* **26** 89 *1957*.] The *3',5'-di-O-acetyl derivative* crystd from aqueous EtOH has **m** 222°(dec), $[\alpha]_D^{18}$ -38° (c 0.3, 10% aq EtOH) [Hayes et al. *J Chem Soc* 808, 813 *1955*].

2'-Deoxyinosine *[890-38-0]* **M 252.2, m 206°(dec), 218-220°(dec), $[\alpha]_D^{25}$ -21° (c 2, N NaOH), $[\alpha]_D^{21.5}$ (c 1, H_2O), $pK_{Est(1)}$~ 8.9, $pK_{Est(2)}$~ 12.4.** Purified by recrystn from H_2O. [Brown and Lythgoe *J Chem Soc* 1990 *1950*; UV: : MacNutt *Biochem J* **50** 384 *1952*.]

5-Deoxy-5-(methylthio)adenosine *[2457-80-9]* **M 297.3, m 210-213°(dec), 211°, 212°, 213-214°, $[\alpha]_D^{20}$ -23.7° (c 0.02, pyridine), $[\alpha]_D^{20}$ -8° (c 1, 5% aq NaOH), $[\alpha]_D^{25}$ +15° (c 0.4-1.0, 0.3N aq AcOH), pK_{Est} ~3.5.** It has been recrystd from H_2O and sublimed at 200°/0.004mm. [v.Euler and Myrbäck *Z physiol Chem* **177** 237 *1928*; Weygand and Trauth *Chem Ber* **84** 633 *1951*; Baddiley et al. *J Chem Soc* 2662 *1953*.] The *hydrochloride* has **m** 161-162° [Kuhn and Henkel *Hoppe Seyler's Z Physiol Chem* **269** 41 *1941*]. The *picrate* has **m** 183°(dec) (from H_2O).

Deoxyribonucleic acid (from plasmids). Purified by two buoyant density ultracentrifugations using ethidium bromide-CsCl. The ethidium bromide was extracted with Et_2O and the DNA was dialysed against buffered EDTA and lyophilised. [Marmur and Doty *J Mol Biol* **5** 109 *1962*; Guerry et al. *J Bacteriol* **116** 1064 *1973*.] See p. 504.

3'-Deoxythymidine {2',3'-dideoxythymidine, 1-[(2*r*)-5*c*-hydroxymethyltetrahydro(2*r*)-furyl]-5-methylpyrimidine-2,4-dione} *[3416-05-5]* **M 226.2, m 145°, 149-151°, $[\alpha]_D^{26}$ +18° (c 1, H_2O), pK_{Est} ~ 9.2.** Recrystd from Me_2CO + MeOH. [Michelson and Todd *J Chem Soc* 816 *1955*.]

2'-Deoxyuridine [1-(β-D-*erythro*-2-deoxypentofuranosyl)-1*H*-pyrimidine-2,4-dione] *[951-78-0]* **M 228.2, m 163°, 163-163.5°, 165-167° 167°, $[\alpha]_D^{26}$ +30° (c 2, H_2O), $[\alpha]_D^{22}$ +50° (c 1, N NaOH), pK^{25} 9.3.** Forms needles from absolute EtOH or 95% EtOH. [Dekker and Todd *Nature* **166** 557 *1950*; Brown et al. *J Chem Soc* 3035 *1958*; NMR Jardetzky *J Am Chem Soc* **83** 2919 *1961*; Fox and Shugar *Biochim Biophys Acta* **9** 369 *1952*; UV: MacNutt *Biochem J* **50** 384 *1952*.]

3'-Deoxyuridine {1-[(2*R*)-5*c*-hydroxymethyltetrahydro(2*r*)furyl]-5-methylpyrimidin-2,4-dione, 2'.3'-dideoxythymidine} *[7057-27-4, 3416-05-5]* **M 226.2, m 149-151°, $[\alpha]_D^{20}$ +18° (c 1, H_2O), pK_{Est} ~ 9.3.** Recrystd from Me_2CO + MeOH and dried in a vacuum. [Michelson and Todd *J Chem Soc* 816 *1955*.]

Dermatan sulfate (condroitin sulfate B from pig skin) *[54328-33-5 (Na salt)]*. Purified by digestion with papain and hyaluronidase, and fractionation using aqueous EtOH. [Gifonelli and Roden *Biochem Prep* **12** 1 *1968*.]

Desthiobiotin *[533-48-2]* **M 214.3, m 156-158°, $[\alpha]_D^{20}$ +10.5° (c 2, H_2O), pK_{Est} ~2.8.** Crystd from H_2O or 95% EtOH.

Dextran *[9004-54-0]* **M_r 6,000-220,000.** Solutions keeps indefinitely at room temperature if 0.2mL of Roccal (10% alkyldimethylbenzylammonium chloride) or 2mg phenyl mercuric acetate are added per 100mL solution. [Scott and Melvin *Anal Biochem* **25** 1656 *1953*.]

Diacetone-D-Glucose (1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranoside) *[582-52-5]* **M 260.3, m 107-110°, 110.5°, 111-113°, 112°, $[\alpha]_D^{15}$ -18.4° (c 1, H_2O).** It crystallises from Et_2O, (needles), pet ether or $^*C_6H_6$ and sublimes *in vacuo*. It is sol in 7 vols of H_2O and 200 vols of pet ether at their

boiling points. The solubility in H_2O at 17.5° is 4.3%. It pptes from aq solns on basification with NaOH. [Schmid and Karrer *Helv Chim Acta* **32** 1371 *1949*; Fischer and Rund *Chem Ber* **49** 90, 93 *1916*; IR: Kuhn *Anal Chem* **22** 276 *1950*.]

***N,N'*-Diacetylchitobiose (2-acetyl-O^4-[2-acetylamino-2-deoxy-β-D-glucopyranosyl]-2-deoxy-D-glucose)** *[35061-50-8]* **M 424.4, m 245-247°(dec), 251.5-252.5°, 260-262°, $[\alpha]_D^{25}$ +39.5° (extrapolated) → +18.5° (after 60 min, c 1, H_2O).** Recrystd from aqueous MeOH or aqueous EtOH + 1,2-dimethoxyethane. [Zilliken et al. *J Chem Soc* **77** 1296 *1955*.]

1,8-Diazafluorenone (cyclopenta[1.2-*b*:4,3-*b'*]dipyridin-9-one) *[54078-29-4]* **M 182.2, m 205°, 229-231°, pK_{Est} ~ 2.6.** Recrystd from Me_2CO. The *oxime* has **m** 119-200°. [Druey and Schmid *Helv Chim Acta* **33** 1080 *1950*.]

Di- and tri-carboxylic acids. Resolution by anion-exchange chromatography. [Bengtsson and Samuelson *Anal Chim Acta* **44** 217b *1969*.]

Digitonin *[11024-24-1]* **M 1229.3, m >270°(dec), $[\alpha]_{546}^{20}$ -63° (c 3, MeOH).** Crystd from aqueous 85% EtOH or MeOH/diethyl ether.

Digitoxigenin *[143-62-4]* **M 374.5, m 253°, $[\alpha]_{546}^{20}$ +21° (c 1, MeOH).** Crystd from aqueous 40% EtOH.

D(+)-Digitoxose *[527-52-6]* **M 148.2, m 112°, $[\alpha]_{546}^{20}$ +57° (c 1, H_2O).** Crystd from MeOH/diethyl ether, or ethyl acetate.

Dihydrofolate reductase (from *Mycobacterium phlei*) *[9002-03-3]* **M_r ~18,000 [EC 1.5.1.3].** Purified by ammonium sulfate pptn, then fractionated on Sephadex G-75 column, applied to a Blue Sepharose column and eluted with 1mM dihydrofolate. [Al Rubeai and Dole *Biochem J* **235** 301 *1986*.]

7,8-Dihydrofolic acid (7,8-dihydropteroyl-L-glutamic acid, DHFA) *[4033-27-6]* **M 443.4, pK_1 2.0 (basic 10-NH), pK_2 2.89 (2-NH_2), pK_3 3.45 (α-CO_2H), pK_4 4.0 (basic 5N), pK_5 4.8 (γ-CO_2H), pK_6 9.54 (acidic 3NH).** Best purified by suspending (1g mostly dissolved)) in ice-cold sodium ascorbate (300mL of 10% at pH 6.0 [prepared by adjusting the pH of 30g of sodium ascorbate in 150mL of H_2O by adding 1N NaOH dropwise using a glass electrode till the pH is 6.0]). This gave a clear solution with pH ~5. While stirring at 0° add N HCl dropwise slowly (0.1mL/min) until the pH drops to 2.8 when white birefringent crystals separate. These are collected by centrifugation (1000xg for 5min), washed 3x with 0.001N HCl by centrifugation and decantation. The residue is then dried in a vacuum (0.02mm) over P_2O_5 (change the P_2O_5 frequently at first) and KOH at 25° in the dark. After 24hours the solid reaches constant weight.
For the assay of *dihydrofolate reductase* (see below): suspend ~66.5mg of DHFA in 10mL of 0.001M HCl containing 10mM dithiothreitol (DTT stock made from 154mg in 10mL H_2O making 0.1M), shake well and freeze in 400μL aliquots. Before use mix 400μL of this suspension with 0.1M DTT (200μL, also made in frozen aliquots), and the mixture is diluted with 200μL of 1.5M Tris-HCl pH 7.0 and 1.2mL of H_2O (making a total volume of ~2mL) to give a clear solution. To estimate the concentration of DHFA in this solution, dilute 20μL of this solution to 1mL with 0.1M Tris-HCl pH 7.0 and read the OD at 282nm in a 1cm pathlength cuvette. ε at 282nm is 28,000$M^{-1}cm^{-1}$. [Reyes and Rathod *Methods Enzymol* **122** 360 *1986*.]

Dihydropteridine reductase (from sheep liver) *[9074-11-7]* **M_r 52,000 [EC 1.6.99.7].** Purified by fractionation with ammonium sulfate, dialysed *versus* tris buffer, adsorbed and eluted from hydroxylapatite gel. Then run through a DEAE-cellulose column and also subjected to Sephadex G-100 filtration. [Craine et al. *J Biol Chem* **247** 6082 *1972*.]

Dihydropteridine reductase (from human liver) *[9074-11-7]* **M_r 52,000 [EC 1.6.99.7].** Purified to homogeneity on a naphthoquinone affinity adsorbent, followed by DEAE-Sephadex and CM-Sephadex

chromatography. [Firgaira, Cotton and Danks, *Biochem J* **197** 31 *1981*.] [For other dihydropteridine reductases see Armarego et al. *Med Res Rev* **4**(3) 267 *1984*.]

DL-*erythro*-Dihydrosphingosine (dl-*erythro*-2-aminooctadecan-1,3-diol) *[3102-56-5]* **M 301.5, m 85-86°, 85-87°, pK_{Est} ~ 8.8.** Purified by recrystn from pet ether-EtOAc or $CHCl_3$. The (±)-*N-dichloroacetyl* derivative has **m** 142-144° (from MeOH). [Shapiro et al. *J Am Chem Soc* **80** 2170 *1958*; Shapiro and Sheradsky *J Org Chem* **28** 2157 *1963*.] The D-isomer crystallises from pet ether-Et_2O and has **m** 78.5-79°, $[\alpha]_{546}^{28}$ +6° ($CHCl_3$ + MeOH, 10:1). [Grob and Jenny *Helv Chim Acta* **35** 2106 *1953*, Jenny and Grob *Helv Chim Acta* **36** 1454 *1953*.]

Dihydrostreptomycin sesquisulfate *[5490-27-7]* **M 461.4, m 250°(dec), 255-265°(dec), $[\alpha]_D^{20}$ -92.4° (c 1, H_2O), $pK_{Est(1)}$~ 9.5 (NMe), $pK_{Est(2,3)}$~ 13.4 (guanidino).** It crystallises from H_2O with MeOH, *n*-BuOH or methyl ethyl ketone. The crystals are not hygroscopic like the amorphous powder, however both forms are soluble in H_2O but the amorphous solid is about 10 times more soluble than the crystals. The *free base* also crystallises from H_2O-Me_2CO and has $[\alpha]_D^{26}$ -92° (aqueous solution pH 7.0). [Solomons and Regina *Science* **109** 515 *1949*; Wolf et al. *Science* **109** 515 *1949*; McGilveray and Rinehart *J Am Chem Soc* **87** *4003* 1956].

3-(3,4-Dihydroxyphenyl)-L-alanine (DOPA, EUODOPA) *[59-92-7, 5796-17-8]* **M 197.2, m 275°(dec), 267-268°(dec), 284-286°(dec), ~295°(dec), $[\alpha]_D^{13}$ -13.1° (c 5.12, N HCl), pK_1^{25} 2.32 (CO_2H), pK_2^{25} 8.72 (NH_2), pK_3^{25} 9.96 (OH), pK_4^{25} 11.79 (OH).** Likely impurities are vanillin, hippuric acid, 3-methoxytyrosine and 3-aminotyrosine. Recryst from large vols of H_2O as colourless white needles; solubility in H_2O is 0.165%, but it is insoluble in EtOH, *C_6H_6, $CHCl_3$, and EtOAc. Also crystd by dissolving in dilute HCl and adding dilute ammonia to give pH 5, under N_2. Alternatively, crystd from dil aqueous EtOH. It is rapidly oxidised in air when moist, and darkens; particularly in alkaline solution. Dry in a vacuum at 70° in the dark, and store in a dark container preferably under N_2. λ_{max} 220.5nm (log ε 3.79) and 280nm (log ε 3.42) in 0.001N HCl. [Yamada et al. *Chem Pharm Bull Jpn* **10** 693 *1962*; Bretschneider et al. *Helv Chim Acta* **56** 2857 *1973*; NMR: Jardetzky and Jardetzky *J Biol Chem* **233** 383 *1958*.]

3,4-Dihydroxyphenylalanine-containing proteins. Boronate affinity chromatography is used in the selective binding of proteins containing 3,4-dihydroxyphenylalanine to a *m*-phenylboronate agarose column and eluting with 1M NH_4OAc at pH 10. [Hankus et al. *Anal Biochem* **150** 187 *1986*.]

3-(3,4-Dihydroxyphenyl)-2-methyl-L-alanine [methyldopa, 2-amino-3-(3,4-dihydroxyphenyl)-2-methylpropionic acid] *[555-30-6]* **M 238.2, m >300°, 300-301°(dec), pK_1^{25} 2.2, pK_2^{25} 9.2, pK_3^{25} 10.6, pK_4^{25} 12.0.** Recrystd from H_2O. [Reinhold et al. *J Org Chem* **33** 1209 *1968*.] The L-*isomer* forms a sesquihydrate from H_2O **m** 302-304° (dec), and the anhydrous crystals are *hygroscopic*, $[\alpha]_D^{23}$ -4.0° (c 1, 0.1N HCl), $[\alpha]_{546}$ +154.5° (c 5, $CuSO_4$ solution). It has λ_{max} 281nm (ε 2780). Solubility in H_2O at 25° is ~10mg/mL and the pH of an aqueous solution is ~5.0. It is almost insoluble in most organic solvents. [Stein et al. *J Am Chem Soc* **77** 700 *1955*.]

(±)-7-(2,3-Dihydroxypropyl)theophylline (Diprophylline, Dyphylline) *[479-18-5]* **M 254.3, m 158°, 160-164°, 161°, 161-164°, pK_{Est} ~ 8.7.** Recrystd from EtOH or H_2O. Solubility in H_2O is 33% at 25°, in EtOH it is 2% and in $CHCl_3$ it is 1%. λ_{max} (H_2O) 273nm (ε 8,855). [Roth *Arch Pharm* **292** 234 *1959*.] The *4-nitrobenzoyl* derivative has **m** 178° [Oshay *J Chem Soc* 3975 *1956*].

3,5-Diiodo-L-thyronine (3,5-diiodo-4-[4-hydroxyphenoxy]-1-phenylalanine) *[1041-01-6]* **M 525.1, m 255°(dec), 255-257°(dec), $[\alpha]_D^{22}$ +26° [2N HCl-EtOH (1:2)], pK_1^{20} 3.25, pK_2^{20} 5.32, pK_3^{20} 9.48.** Recrystd from EtOH. [Chambers et al. *J Chem Soc* 3424 *1949*.]

3,5-Diiodo-L-tyrosine dihydrate *[300-39-0]* **M 469.0, m 199-210°, 202°(dec), $[\alpha]_D^{20}$ +2.89° (c 4.9, 4% HCl), pK_1^{25} 2.12, pK_2^{25} 6.48, pK_3^{25} 7.82.** It forms crystals from H_2O [solubility (g/L): 0.204 at 0°, 1.86 at 50°, 5.6 at 75° and 17.0 at 100°]. Also recrysts from 50% or 70% EtOH. When boiled in EtOH the crystals swell and on further boiling a gelatinous ppte is formed [Harrington *Biochem J* **22** 1434 *1928* ; Jurd *J Am Chem Soc* **77** 5747 *1955*]. Also crystd from cold dilute ammonia by adding acetic acid to pH 6.

1,2-Dilauroyl-sn-glycero-3-phosphoethanolamine (±-dilauroyl-α-cephalin, 3-sn-phosphatidylethanolamine 1,2-didodecanoyl) *[59752-57-7]* **M 579.8, m 210°, $pK_{Est(1)}$~ 5.8 (PO_4H), $pK_{Est(2)}$~ 10.5 (NH_2).** Recrystd from EtOH or tetrahydrofuran. [Bevan and Malkin *J Chem Soc* 2667 *1951*; IR: Bellamy and Beecher *J Chem Soc* 728 *1953*.]

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride [1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride] see entry on p. 212 in Chapter 4.

1,2-Dimyristoyl-sn-glycero-3-phosphocholine monohydrate (dimyristoyl-L-α-lecithin) *[18194-24-6]* **M 696.0, $[\alpha]_D^{24}$ +7° (c 8, EtOH-$CHCl_3$ 1:1 for α_1 form), pK_{Est} ~ 5.8 (PO_4).** Three forms α_1, α_2 and β'. Recrystd from aqueous EtOH or EtOH-Et_2O. Solubility at 22-23° in Et_2O is 0.03%, in Me_2CO it is 0.06% and in pyridine it is 1.3%. [Baer and Kates *J Am Chem Soc* **72** 942 *1950*; Baer and Maurakas *J Am Chem Soc* **74** 158 *1952*; IR: Marinetti and Stotz *J Am Chem Soc* **76** 1347 *1954*.] The *S-isomer* with 1 H_2O is recrystd from 2,6-dimethylheptan-4-one and has **m** 226-227° (sintering at 90-95°), and $[\alpha]_D$ -7° (c 6, MeOH-$CHCl_3$ 1:1). [Baer and Martin *J Biol Chem* **193** 835 *1951*.]

(±)-1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine (dimyristoyl-α--kephalin) *[998-07-2]* **M 635-9, m 207°, $pK_{Est(1)}$~ 5.8 (PO_4H), $pK_{Est(2)}$~ 10.5 (NH_2).** Recrystd from EtOH [Bevan and Malkin *J Chem Soc* 2667 *1951*]. The *R-isomer* has **m** 195-196° (sintering at 130-135°) after recrystn from $CHCl_3$-MeOH, $[\alpha]_D^{26}$ +6.7° (c 8.5, $CHCl_3$-AcOH 9:1). [Baer *Can J Biochem Physiol* **35** 239 *1957*; Baer et al. *J Am Chem Soc* **74** 152 *1952*.]

***S*-1,2-Dipalmitin** *[761-35-3]* **M 568.9, m 68-69° $[\alpha]_D^{20}$-2.9° (c 8, $CHCl_3$), $[\alpha]_{546}^{20}$ +1.0° (c 10, $CHCl_3$/MeOH, 9:1).** Crystd from chloroform/pet ether.

***R*-Dipalmitoyl-sn-glycero-3-phosphatidic acid** *[7091-44-3]* **M 648.9, $[\alpha]_D^{26}$ + 4 ° (c 10, $CHCl_3$), $pK_{Est(1)}$~ 1.6, $pK_{Est(2)}$~ 6.1.** Recrystd from Me_2CO at low temp. At 21° it is soluble in *C_6H_6 (4.2%), pet ether (0.01%), MeOH (2%), EtOH (2.5%), AcOH (1.3%), Me_2CO (1.76%), and Et_2O (1.5%). [Baer *J Biol Chem* **189** 235 *1951*.]

***R*-1,2-Dipalmitoyl-sn-glycero-3-phosphocholine monohydrate (dipalmitoyl-α-L-lecithin)** *[63-89-8]* **M 752.1, m sinters at 120°, $[\alpha]_D^{25}$ +7.0° (c 5.6, abs $CHCl_3$), pK_{Est} ~ 5.8 (PO_4).** It has three crystn forms α_1, α_2 and β' which change at 60-70° and at 229° respectively. In order to obtain a fine powder, ~2 g are dissolved in $CHCl_3$ (15mL) and pet ether (b 35-60°) is added and the soln evaporated to dryness *in vacuo* <20°, and then dried at 0.1mm over $CaCl_2$. [Baer and Maurukas *J Am Chem Soc* **74** 158 *1952*; Baer and Kates *J Biol Chem* **185** 615 *1950*.]

***d,l*-βγ-Dipalmitoylphosphatidyl choline** *[2797-68-4]* **M 734.1, m 230-233°, pK_{Est} ~ 5.8 (PO_4).** Recrystd from chloroform and dried for 48h at 10^{-5} torr [O'Leary and Levine *J Phys Chem* **88** 1790 *1984*].

Dipeptidyl aminopeptidase (from rat brain) *[9031-94-1]* **[EC 3.4.11.10].** Purified about 2000-fold by column chromatography on CM-cellulose, hydroxylapatite and Gly-Pro AH-Sepharose. [Imai et al. *J Biochem (Tokyo)* **93** 431 *1983*.]

1,2-Distearoyl-sn-glycerol *[1429-59-0]* **M 625.0.** The dl-form recrystallises from $CHCl_3$-pet ether (b 40-60°), **m** 59.5° (α form) and 71.5-72.5° (β form). Recrystn from solvents (e.g. EtOH, MeOH, toluene, Et_2O) gives the higher melting form and resolidification gives the lower melting forms. [IR: Chapman *J Chem Soc* 4680 *1958*, 2522 *1956*; .] The *S-isomer* is recrystd from $CHCl_3$-pet ether and has **m** 76-77°, $[\alpha]_D^{24}$ -2.8° (c 6, $CHCl_3$). [Baer and Kates *J Am Chem Soc* **72** 942 *1950*.]

1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (distearoyl-α-kephalin) *[1069-79-0]* **M 748.1, m 180-182° (*R*-form, sintering at 130-135°), m 196° (± form), $pK_{Est(1)}$~ 5.8 (PO_4H), $pK_{Est(2)}$~ 10.5 (NH_2).** The *R*-form is recrystd from $CHCl_3$-MeOH and the ±-form is recrystd from EtOH. [Bevan and Malkin *J Chem Soc* 2667 *1951*; Baer *Can J Biochem Physiol* **81** 1758 *1959*.]

Dolichol (from pig liver) *[11029-02-0]* **C_{80}-C_{105} polyprenols.** Cryst 6 times from pet ether/EtOH at -20°C. Ran as entity on a paper chromatogram on paraffin impregnated paper, with acetone as the mobile phase. [Burgos *Biochem J* **88** 470 *1963.*]

Domoic acid [4-(2-carboxyhexa-3,5-dienyl)-3-carboxymethylproline] *[14277-97-5]* **M 311.3, m 215°, 217°, $[\alpha]_D^{20}$ -108° (c 1,H_2O), pK_1 2.20 (2-CO_2H), pK_2 3.72 (CO_2H), pK_3 4.93 (3-CH_2CO_2H), pK_4 9.82 (NH).** The acid (~300 mg) is purifed on a Dowex 1 column (3.5 x 40 mm, 200-400 mesh, acetate form), washed with H_2O until neutral, then eluted with increasing concentrations of AcOH (8L) from 0 to 0.25M. The fraction containing domoic acid (in 50mL) is collected, evaporated to dryness under reduced pressure and recrystd from aqueous EtOH. Glutamate and Kainate receptor agonist. [Impellizzeri et al. *Phytochemistry* **14** 1549 *1975*; Takemoto and Diago *Arch Pharm* **293** 627 *1960.*]

DNA (deoxyribonucleic acids). The essential structures of chromosomes are DNA and contain the genetic "blue print" in the form of separate genes. They are made up of the four deoxyribonucleic acids (nucleotides): adenylic acid, guanylic acid, cytidylic acid and thymidylic acid (designated A, G, C, T respectively) linked together by their phosphate groups in ester bonds between the 3' and 5' hydroxy groups of the 2'-deoxy-D-ribose moiety of the nucleotides. The chains form a double stranded spiral (helix) in which the two identical nucleotide sequences run antiparallel with the heterocyclic bases hydrogen bonded (A..T, G..C) forming the "ladder" between the strands. Short sequences of DNA are available commercially, are commercially custom made or synthesised in a DNA synthesiser and purified by HPLC. Their purity can be checked by restriction enzyme cleavage followed by gel electophoresis, or directly by gel electrophoresis or analytical HPLC. Commercial DNAs are usually pure enough for direct use but can be further purified using commercially available kits involving binding to silica or other matrices and eluting with tris buffers.

Dopamine-β-hydroxylase (from bovine adrenal medulla) *[9013-38-1]* **M_r ~290,000, [EC 1.14.17.1].** The Cu-containing glycoprotein enzyme has been isolated by two procedures. The first is an elaborate method requiring extraction, two $(NH_4)_2SO_4$ fractionations, calcium phosphate gel filtration, EtOH fractionation, DEAE-cellulose chromatography followed by two Sephadex-G200 gel filtrations giving enzyme with a specific activity of 65 Units/mg. [Friedman and Kaufman *J Biol Chem* **240** 4763 *1965*; Rush et al. *Biochem Biophys Res Commun* **61** 38 *1974.*] The second procedure is much gentler and provides good quality enzyme. Sedimented chromaffin vesicles were lysed in 10 volumes of 5mM K-phosphate buffer pH 6.5 using a loosely fitting Teflon-glass homogeniser. The mixture is centrifuged at 40,000xg/0.5 h and the supernatant is diluted with an equal volume of 100mM phosphate buffer (pH 6.5) containing 0.4M NaCl. This lysate is applied to a concanavelin A-Sepharose column (4 x 0.7cm) which had been equilibrated with 50 mM of phosphate buffer (pH 6.5 + 0.2M NaCl) with a flow rate of ~ 0.3 mL/min. The column is washed thoroughly with the buffer until OD_{280nm} is 0.005. The enzyme is then eluted with the same buffer containing 10% α-methyl-D-mannoside (flow rate 0.1 mL/min) and the enzyme is collected in twenty column volumes. The pooled eluate is concentrated by ultrafiltration in an Amicon Diaflo stirrer cell using an XM100A membrane. The concentrated enzyme is dialysed against 50 mM phosphate buffer (pH 6.5) containing 0.1% NaCl. The enzyme gives one band (+ two very weak band) on disc gel electrophoresis indicating better than 93% purity (67% fold purification) and has a specific activity of 5.4Units/mg. [Rush et al. *Biochem Biophys Res Commun* **57** 1301 *1974*; Stewart and Klinman *Ann Rev Biochem* **57** 551 *1988.*]

Ellipticine (5,11-dimethylpyrido[4,3:*b*]carbazole) *[519-23-3]* **M 246.3, m 311-315°(dec), 312-314°(dec), pK 5.78 (80% aq methoxyethanol).** This DNA intercalator is purified by recrystn from $CHCl_3$ or MeOH and dried *in vacuo.* The UV λ_{max} values in aqueous EtOH-HCl are at 241, 249, 307, 335 and 426nm. [Marini-Bettolo and Schmutz *Helv Chim Acta* **42** 2146 *1959.*] The *methiodide* has **m** 360°(dec), with UV λ_{max} (EtOH-KOH) 223, 242, 251, 311, 362 and 432nm. [Goodwin et al. *J Am Chem Soc* **81** 1903 *1959.*]

Enniatin A *[11113-62-5]* **M 681.9, m 122-122.5°, $[\alpha]_D^{18}$ -92° (c 0.9, $CHCl_3$).** A cyclic peptidic ester antibiotic which is recrystd from EtOH/water but is deactivated in alkaline soln. [Ovchinnikov and Ivanov in *The Proteins* (Neurath and Hill eds) Academic Press, NY, Vol V pp. 365 and 516 *1982.*]

(-)-Ephedrine (1*R*,2*S*-2-methylamino-1-phenylpropanol) *[299-42-3]* **M 165.2, m ~34°, 36°, 38.1°, b 126-129°/7mm, 225-227°/760mm, d^{22} 1.0085, $[\alpha]_D^{26}$ -42° (c 4, 3% HCl), $[\alpha]_D^{22.5}$ +15.1° (c 0.8, H_2O), -9.36° (c 3, MeOH), pK^{22} 9.58 (pK^{25} 8.84 in 80% aq methoxy-ethanol).** Purified by vacuum distn (dehydrates) and forms waxy crystals or granules, and may pick up 0.5 H_2O. The presence of H_2O raises its **m** to 40°. [Moore and Taber *J Amer Pharm Soc* **24** 211 *1935.*] The anhydrous base recrystd from dry ether [Fleming and Saunders *J Chem Soc* 4150 *1955*]. It gradually decomposes on exposure to light and is best stored in an inert atmosphere in the dark (preferably at -20°). Sol in H_2O is 5%, in EtOH it is 1% and it is soluble in $CHCl_3$, Et_2O and oils. It has pKa values in H_2O of 10.25 (0°) and 8.69 (60°) [Everett and Hyne *J Chem Soc* 1136 *1958*; Prelog and Häflinger *Helv Chim Acta* **33** 2021 *1950*] and pKa^{25} 8.84 in 80% aqueous methoxyethanol [Simon *Helv Chim Acta* **41** 1835 *1958*]. The *hydrochloride* has **m** 220° (from EtOH-Et_2O) and $[\alpha]_D^{20}$ -38.8° (c 2, EtOH). [IR: Chatten and Levi *Anal Chem* **31** 1581 *1959.*] The anhydrous base crystallises from Et_2O [Fleming and Saunders *J Chem Soc* 4150 *1955*].

(+)-Ephedrine hydrochloride (1*S*-2*R*-2-methylamino-1-phenylpropan-1-ol hydrochloride) *[24221-86-1]* **M 201.7, m 216-219°, $[\alpha]_D^{20}$ +34° (c 11.5, H_2O).** Recrystd from EtOH-Et_2O. The *free base* recrystallises from *C_6H_6 with **m** 40-41° (Skita et al. *Chem Ber* **66** 974 *1933*].

Erythromycin A *[114-07-8]* **M 733.9, m 133-135°(dec), 135-140°, 137-140°, $[\alpha]_D^{20}$ -75° (c 2, EtOH), pK 8.9.** It recrystallises from H_2O to form hydrated crystals which melt at *ca* 135-140°, resolidifies and melts again at 190-193°. The **m** after drying at 56°/8mm is that of the **anhydrous** material at 137-140°. Its solubility in H_2O in ~2mg/mL. The *Hydrochloride* has **m** 170°, 173° (from aq EtOH, EtOH-Et_2O). [Flynn et al. *J Am Chem Soc* **76** 3121 *1954*; constitution : Wiley et al. *J Am Chem Soc* **79** 6062 *1957*].

β-Estradiol (1,3,5-estratrien-3,17β-diol) *[50-28-2]* **M 272.4, m 173-179°, 176-178°, $[\alpha]_D^{20}$ +76° to +83° (c 1, dioxane).** Purified by chromatography on SiO_2 (toluene-EtOAc 4:1) and recrystd from $CHCl_3$-hexane or 80% EtOH. It is stable in air and insoluble in H_2O and is ppted by digitonin. UV λ_{max} at 225 and 280 nm. [Oppolzer and Roberts *Helv Chim Acta* **63** 1703 *1980.*]

β-Estradiol-6-one (1,3,5-estratriene-3,17β-diol-6-one) *[571-92-6]* **M 359.4, m 278-280°, 281-283°, $[\alpha]_D^{20}$ +4.2° (c 0.7, EtOH).** It forms plates from EtOH. The *3,17-diacetate* has **m** 173-175° after recrystn from aqueous EtOH. [Longwell and Wintersteiner *J Biol Chem* **133** 219 *1940.*] The UV has λ_{max} 255 and 326nm in EtOH [Slaunwhite et al. *J Biol Chem* **191** 627 *1951*].

Ethidium bromide *[1239-45-8]* **M 384.3, m 260-262°.** Crystd from MeOH or EtOH [Lamos et al. *J Am Chem Soc* **108** 4278 *1986*]. Sol in H_2O is 1%. **POSSIBLE CARCINOGEN.**

Ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) *[91-53-2]* **M 217.3, b 169°/12-13mm, d_4^{20} 1.000, pK_{Est} ~ 5.8.** Purified by fractional distn *in vacuo* and solidifies to a glass. [Knoevenagel *Chem Ber* **54** 1723, 1730 *1921*]. The *methiodide* has **m** 179° (from EtOH) and the *1-phenylcarbamoyl* derivative has **m** 146-147° (from EtOH). [Beaver et al. *J Am Chem Soc* **79** 1236 *1957.*]

17-α-Ethynylestradiol *[57-63-6]* **M 296.4, m 141-146°, 145-146°, $[\alpha]_D^{20}$ +4° (c 1, $CHCl_3$).** It forms a hemihydrate on recrystn from MeOH-H_2O. It dehydrated on melting and re-melts on further heating at **m** 182-184°. UV λ_{max} at 281nm (ε 2040) in EtOH. Solubility is 17% in EtOH, 25% in Et_2O, 20% in Me_2CO, 25% in dioxan and 5% in $CHCl_3$. [Petit and Muller *Bull Soc Chim Fr* 121 *1951.*] The *diacetyl* derivative has **m** 143-144° (from MeOH) and $[\alpha]_D^{20}$ +1° (c 1, $CHCl_3$) [Mills et al. *J Am Chem Soc* **80** 6118 *1958*].

Exonucleases. Like the endonucleases they are restriction enzymes which act at the 3' or 5' ends of linear DNA by hydrolysing off the nucleotides. Although they are highly specific for hydrolysing nucleotides at the 3' or 5' ends of linear DNA, the number of nucleotides cleaved are time dependent and usually have to be estimated from the time allocated for cleavage. Commercially available exonucleases are used without further purification.

Farnesol **(*trans-trans*-3,7,11-trimethyl-2,6,10-dodecatrien-1-ol)** *[106-28-5]* **M 222.4, b 111°/0.35mm, 126-127°/0.5mm, 142-143°/2mm, d_4^{20} 0.8871, n_D^{25} 1.4870.** Main impurity is the *cis-trans* isomer. Purified by gas chromatography using a 4ft x 0.125in 3%OV-1 column at 150°. [Corey et al. *J Am Chem Soc* **92** 6637 *1970*; Popjak et al. *J Biol Chem* **237** 56 *1962*.] Also purifed through a 14-in Podbielniak column at 11°/0.35mm (see p. 141). Alternatively it has been purified by gas chromatography using SF96 silicone on Fluoropak columns or Carbowax 20M on Fluoropak or base-washed 30:60 firebrick (to avoid decomp of alcohol, prepared by treating the firebrick with 5N NaOH in MeOH and washed with MeOH to pH 8) at 210° with Helium carrier gas at 60 mL/min flow rate. The *diphenylcarbamoyl* derivative has **m** 61-63° (from MeOH) and has IR band at 3500 cm^{-1}. [Bates et al. *J Org Chem* **28** 1086 *1963*.]

Farnesyl pyrophosphate *[13058-04-3; E,E: 372-97-4]* **M 382.3, $pK_{Est(1)}$~<2, $pK_{Est(2)}$~<2, $pK_{Est(3)}$~3.95, $pK_{Est(4)}$~6.26.** Purified by chromatography on Whatman No3 MM paper in a system of isopropanol-isobutanol-ammonia-water (40:20:1:30) (v/v). Stored as the Li or NH_4 salt at 0°.

Ferritin (from human placenta) *[9007-73-2]* **M_r ~445,000 (Fe free protein).** The purification of this major iron binding potein was achieved by homogenisation in water and precipitating with ammonium sulfate, repeating the cycle of ultracentrifuging and molecular sieve chromatography through Sephadex 4B column. Isoelectric focusing revealed a broad spectrum of impurities which were separated by ion-exchange chromatography on Sephadex A-25 and stepwise elution. [Konijn et al. *Anal Biochem* **144** 423 *1985*.]

Fibrinogen (from human plasma) *[9001-32-5]* **M_r 341,000.** A protein made up of 2Aα,2Bβ and 2γ subunits connected by disulfide bridges. Possible impurity is plasminogen. Purified by glycine pptn [Mosesson and Sherry *Biochemistry* **5** 2829 *1966*] to obtain fractions 1-2, then further purified [Blombäck and Blombäck *Arkiv Kemi* **10** 415 *1956*] and contaminating plasminogen is removed by passage through a lysine-Sepharose column. Such preparations were at least 95% clottable as determined by Mosesson and Sherry's method (above ref.) in which the OD_{280} was measured before and after clotting with 5 Units/mL of thrombin (> 3000U/mg). All fibrinogen preps were treated with calf intestinal alkaline phosphatase to convert any fibrinogen peptide-AP to fibrinogen peptide-A by removing serine-bound phosphate. Solutions are then lyophilised and stored at -20°. [Higgins and Shafer *J Biol Chem* **256** 12013 *1981*.] It is sparingly soluble in H_2O. Aqueous solns are viscous with isoelectric point at pH 5.5. Readily denatured by heating above 56° or by chemical agents, e.g. salicylaldehyde, naphthoquinone sulfonates, ninhydrin or alloxan. [Edsall et al. *J Am Chem Soc* **69** 2731 *1947*; Purification: Cama et al. *Naturwissenschaften* **48** 574 *1961*; Lorand and Middlebrook *Science* **118** 515 *1953*; *cf.* Fuller in *Methods Enzymol* **163** 474 *1988*.]
For plasminogen-deficient fibrinogen from blood plasma, the anticoagulated blood was centrifuged and the plasma was frozen and washed with saline solution. Treated with charcoal and freeze-thawed. Dialysed *versus* Tris/NaCl buffer. [Maxwell and Nikel *Biochem Prep* **12** 16 *1968*.]

Fibronectin (from human plasma) *[86088-83-7]* **M_r ~220,000.** This glycoprotein contains 5-12% of carbohydrate. It has been purified by glycine fractionation and DEAE-cellulose chromatography. Material is dissolved in 0.25M Tris-phosphate buffer pH 7.0, diluted to 20% and glycine added gradually till 2.1M when the temperature falls to below 15°. The ppte contains mainly fibrinogen. The supernatant is discarded and the ppte is treated with an equal volume of H_2O, cooled (to 0°) and ppted by adding EtOH to 16% (v/v) at -4°. The ppte contains some CI (Cold Insoluble) globulin, fibronectin and small quantities of other proteins. To remove these the ppte is dissolved in 0.25M Tris-phosphate buffer (pH 7.0) *ca* 0.5% and purified by DEAE-cellulose chromatography after diluting the buffer to 0.05M buffer. [Morrison et al. *J Am Chem Soc* **70** 3103 *1948*; Mosesson and Umfleet *J Biol Chem* **245** 5728 *1970*; Mosesson and Amrani *Blood* **56** 145 *1980*; Akiyama and Yamada *Adv Enzymol* **59** 51 *1987*.]

Flavin adenine dinucleotide (di-Na, $2H_2O$ salt, FAD) *[146-14-5]* **M 865.6, $[\alpha]_{546}$ -54° (c 1, H_2O).** Small quantities, purified by paper chromatography using *tert*-butyl alcohols/water, cutting out the main spot and eluting with water. Larger amounts can be ppted from water as the uranyl complex by adding a slight excess of uranyl acetate to a soln at pH 6.0, dropwise and with stirring. The soln is set aside overnight in the cold, and the ppte is centrifuged off, washed with small portions of cold EtOH, then with cold, peroxide-free diethyl ether. It is dried in the dark under vacuum over P_2O_5 at 50-60°. The uranyl complex is suspended in water and, after adding sufficient 0.01M NaOH to adjust the pH to 7, the ppte of uranyl hydroxide is removed by

centrifugation [Huennekens and Felton *Methods Enzymol* 3 954 *1957*]. It can also be crystd from water. Should be kept in the dark. More recently it was purified by elution from a DEAE-cellulose (Whatman DE 23) column with 0.1M phosphate buffer pH 7, and the purity was checked by TLC. [Holt and Cotton, *J Am Chem Soc* **109** 1841 *1987*.]

Flavin mononucleotide (Na, $2H_2O$ salt, FMN) *[130-40-5]* **M 514.4, pK_1 2.1 (PO_4H_2), pK_2 6.5 (PO_4H^-), pK_3 10.3 (CONH), fluorescence λmax 530nm (870nm for reduced form).** Purified by paper chromatography using *tert*-butanol-water, cutting out the main spot and eluting with water. Also purified by adsorption onto an apo-flavodoxin column, followed by elution and freeze drying Crystd from acidic aqueous soln. [Mayhew and Strating *Eur J Biochem* **59** 539 *1976*.]

4-Fluoro-7-nitrobenzofurazan (4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole) *[29270-56-2]* **M 183.1, m 52.5-53.5°, 53-56°, 53.5-54.5°.** Purified by repeated recrystn from pet ether (b 40-60°). On treatment with MeONa in MeOH it gave *4-methoxy-7-nitrobenzo-2-oxa-1,3-diazole* **m** 115-116°. [Nunno et al. *J Chem Soc (C)* 1433 *1970*.] It is a very good fluorophore for amino acids [Imai and Watanabe *Analyt Chim Acta* **130** 377 *1981*], as it reacts with primary and secondary amines to form fluorescent adducts with λ_{ex} 470nm and λ_{em} 530nm. It gives a *glycine derivative* with **m** 185-187° [Miyano et al. *Anal Chim Acta* **170** 81 *1985*].

4-Fluoro-3-nitrophenylazide *[28166-06-5]* **M 182.1, m 53-55°, 54-56°.** Dissolve in Et_2O, dry over $MgSO_4$, filter, evaporate and recryst the residue from pet ether (b 20-40°) to give orange needles. Store in a stoppered container at ~0°. The NMR has δ 7.75 (m 1H) and 7.35 (m 2H) in $CDCl_3$. [Hagedorn et al. *J Org Chem* **43** 2070 *1978*.]

2-Fluorophenylalanine *[R(+)- 97731-02-7; S(-)- 19883-78-4]* **M 183.2, m 226-232°, 231-234°, $[\alpha]_D^{25}$(+) and (-) 15° (c 2, H_2O pH 5.5), pK_1^{24} 2.12, pK_2^{24} 9.01.** Recryst from aqueous EtOH. The *hydrochloride* has **m** 226-231°(dec), and the *N-acetyl* derivative has **m** 147-149° (from aqueous EtOH). [Bennett and Nieman *J Am Chem Soc* **72** 1800 *1950*.]

4-Fluorophenylalanine *[R(+)- 18125-46-7; S(-)- 1132-68-9]* **M 183.2, m 227-232°, $[\alpha]_D^{25}$ (+) and (-) 24° (c 2, H_2O), pK_1^{24} 2.13, pK_2^{24} 9.05.** It is recrystd from aqueous EtOH. The R-*N-acetyl* derivative has **m** 142-145°, $[\alpha]_D^{25}$ -38.6° (c 8, EtOH). [Bennett and Nieman *J Am Chem Soc* **72** 1800 *1950*.]

5-Fluoro-L-tryptophan monohydrate *[16626-02-1]* **M 240.2, m >250°(dec), $[\alpha]_D^{20}$ +5.5° (c 1, 0.1N HCl), $pK_{Est(1)}$~ 2.5 (CO_2H), $pK_{Est(2)}$~ 9.4 (NH_2), $pK_{Est(3)}$~16 (indole-NH).** Recrystd from aqueous EtOH.

5-Fluorouridine (5-fluoro-1-β-D-ribofuranosyl-1*H*-pyrimidine-2,4-dione) *[316-46-1]* **M 262.2, m 180-182°, 182-184°, $[\alpha]_D^{20}$ +18° (c 1, H_2O), $pK_{Est(1)}$~ 8.0, $pK_{Est(2)}$~ 13.** Recrystd from EtOH-Et_2O and dried at 100° in a vacuum. UV: λ_{max} 269nm (pH 7.2, H_2O), 270nm (pH 14, H_2O). [Liang et al. *Mol Pharmacol* **21** 224 *1982*.]

5-Fluorouracil (5-fluoropyrimidinedi-2,4-[1*H*,3*H*]-one) *[51-21-8]* **M 130.1, m 282-283°(dec), 282-286°(dec), pK_1^{25} 8.04, pK_2^{25} 13.0.** Recrystd from H_2O or MeOH-Et_2O, and sublimed at 190-200°/0.1mm or 210-230°/0.5mm. UV: λ_{max} 265-266nm (ε 7070). [Barton et al. *J Org Chem* **37** 329 *1972*; Duschinsky and Pleven *J Am Chem Soc* **79** 4559 *1957*.]

Fluram (Fluorescamine, 4-phenyl-spiro[furan-2(3*H*)-1-phthalan]-3,3'-dione) *[38183-12-9]* **M 278.3, m 153-155°, 154-155°.** A non-fluorescent reagent that reacts with primary amines to form highly fluorescent compounds. Purified by dissolving (~1g) in Et_2O-$^*C_6H_6$ (1:1, 180 mL), wash with 1% aq $NaHCO_3$ (50mL), dry (Na_2SO_4), evaporate in a vacuum. Dissolve the residue in warm CH_2Cl_2 (5mL), dilute with Et_2O (12mL) and refrigerate. Collect the solid and dry in a vacuum. IR ($CHCl_3$): ν 1810, 1745, 1722, 1625 and 1600 cm^{-1}, and NMR ($CDCl_3$): δ 8.71 (s, -OHC=). [Weigele et al. *J Am Chem Soc* **94** 5927 *1972*, *J Org Chem* **41** 388 *1976*; *Methods Enzymol* **47** 236 *1977*.]

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*.]

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