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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*.]

Papain *[9001-73-4]* **M_r ~21,000, [EC 3.4.22.2], amorphous.** A suspension of 50g of papain (freshly ground in a mortar) in 200mL of cold water was stirred at 4° for 4h, then filtered through a Whatman No 1 filter paper. The clear yellow filtrate was cooled in an ice-bath while a rapid stream of H_2S was passed through it for 3h, and the suspension was centrifuged at 2000rpm for 20min. Sufficient cold MeOH was added slowly and with stirring to the supernatant to give a final MeOH concn of 70 vol%. The ppte, collected by centrifugation for 20min at 2000rpm, was dissolved in 200mL of cold water, the soln was saturated with H_2S, centrifuged, and the enzyme again ppted with MeOH. The process was repeated four times. [Bennett and Niemann *J Am Chem Soc* **72** 1798 *1950*.] Papain has also been purified by affinity chromatography on a column of Gly-Gly-Tyr-Arg-agarose [Stewart et al. *J Am Chem Soc* **109** 3480 *1986*].

Papaverine hydrochloride (6,7-dimethoxy-1-veratrylisoquinoline hydrochloride) *[61-25-6]* **M 375.9, m 215-220°, 222.5-223.5°(dec), 231°, pK^{25} 6.41.** Recrystd from H_2O and sublimed at 140°/0.1mm. Solubility in H_2O is 5%. [Saunders and Srivastava *J Pharm Pharmacol* **3** 78 *1951*; Biggs *Trans Faraday Soc* **50** 800 *1954*.] The *free base* has **m** 148-150° [Bobbitt *J Org Chem* **22** 1729 *1957*].

Pargyline hydrochloride (Eutonyl, *N*-methyl-*n*-propargylbenzylamine hydrochloride) *[306-07-0]* **M 195.7, m 154-155°, 155°, pK^{25} 6.9.** Recrystd from EtOH-Et_2O and dried *in vacuo*. It is very soluble in H_2O, in which it is unstable. The *free base* has **b** 101-103°/11mm. It is a glucuronyl transferase inducer and a monoamine oxidase inhibitor. [von Braun et al. *Justus Liebigs Ann Chem* **445** 205 *1928*; Yeh and Mitchell *Experientia* **28** 298 *1972*; Langstrom et al. *Science* **225** 1480 *1984*.]

Pectic acid *[9046-40-6]* **M_r $(C_6H_8O_6)_n$ ~500,000, amorphous, $[\alpha]_D$ +250° (c 1, 0.1M NaOH).** Citrus pectic acid (500g) was refluxed for 18h with 1.5L of 70% EtOH and the suspension was filtered hot. The residue was washed with hot 70% EtOH and finally with ether. It was dried in a current of air, ground and dried for 18h at 80° under vacuum. [Morell and Link *J Biol Chem* **100** 385 *1933*.] It can be further purified by dispersing in water and adding just enough dilute NaOH to dissolve the pectic acid, then passing the soln through columns of cation- and anion-exchange resins [Williams and Johnson *Ind Eng Chem (Anal Ed)* **16** 23 *1944*], and precipitating with two volumes of 95% EtOH containing 0.01% HCl. The ppte is worked with 95% EtOH, then Et_2O, dried and ground.

Pectin *[9000-69-5]* **M_r 25,000-100,000, amorphous.** Dissolved in hot water to give a 1% soln, then cooled, and made about 0.05M in HCl by addition of conc HCl, and ppted by pouring slowly, with vigorous stirring into two volumes of 95% EtOH. After standing for several hours, the pectin is filtered onto nylon cloth, then redispersed in 95% EtOH and stood overnight. The ppte is filtered off, washed with EtOH/Et_2O, then Et_2O and air dried.

D-(-)-Penicillamine (*R*-3-mercapto-D-valine, 3,3-dimethyl-D-cysteine, from natural penicillin) *[52-67-5]* **M 149.2, m 202-206°, 214-217°, $[\alpha]_D^{21}$ -63° (c 1, N NaOH or pyridine), pK_1^{20} 2.4 (CO_2H), pK_2^{20} 8.0 (SH), pK_3^{20} 10.68 (NH_2).** The melting point depends on the rate of heating (**m** 202-206° is obtained by starting at 195° and heating at 2°/min). It is soluble in H_2O and alcohols but insoluble in Et_2O, $CHCl_3$, CCl_4 and hydrocarbon solvents. Purified by dissolving in MeOH and adding Et_2O slowly. Dried *in vacuo* and stored under N_2. [Weight et al. *Angew Chem, Int Ed Engl* **14** 330 *1975*; Cornforth in *The Chemistry of Penicillin* (Clarke, Johnson and Robinson Eds) Princeton Univ Press, 455 *1949*; Polymorphism: Vidler *J Pharm Pharmacol* **28** 663 *1976*.] The D-S-*benzyl derivative* has **m** 197-198° (from H_2O), $[\alpha]_D^{17}$ -20° (c 1, NaOH), -70° (N HCl).

L-(-)-Penicillamine *[1113-41-3]* **M 149.2, m 190-194°, 202-206°, 214-217°, $[\alpha]_D^{21}$ +63° (c 1, N NaOH or pyridine).** Same as preceding entry for its enantiomer.

D-Penicillamine disulfide hydrate (*S,S'*-di-[D-penicillamine] hydrate) *[20902-45-8]* **M 296.4 + aq, m 203-204°(dec), 204-205°(dec), $[\alpha]_D^{23}$ +27° (c 1.5, N HCl), -82° (c 0.8, N NaOH), $pK_{Est(1)}$~ 2.4 (CO_2), $pK_{Est(2)}$~ 10.7 (NH_2).** Purified by recrytn from EtOH or aqueous EtOH. [Crooks in *The Chemistry of Penicillin* (Clarke, Johnson and Robinson Eds) Princeton Univ Press, 469 *1949*; Use as a thiol reagent for proteins: Garel *Eur J Biochem* **123** 513 *1982*; Süs *Justus Liebigs Ann Chem* **561** 31 *1948*.]

Pepsin *[9001-75-6]* **M_r 31,500(human), 6000(hog) [EC 3.4.23.1].** Rechromatographed on a column of Amberlite CG-50 using a pH gradient prior to use. Crystd from EtOH. [Richmond et al. *Biochim Biophys Acta* **29** 453 *1958*; Huang and Tang, *J Biol Chem* **244** 1085 *1969*, **245** 2189 *1970*.]

Pertussis toxin (from *Bordetella pertussis*) *[70323-44-3]* **M_r 117,000.** Purified by stepwise elution from 3 columns comprising Blue Sepharose, Phenyl Sepharose and hydroxylapatite, and SDS-PAGE [Svoboda et al. *Anal Biochem* **159** 402 *1986*; *Biochemistry* **21** 5516 *1982*; *Biochem J* **83** 295 *1978*.]

2-Phenylethyl-β-D-thiogalactoside *[63407-54-5]* **M 300.4, m 108°, $[\alpha]_D^{23}$ -32.2° (c 5, MeOH).** Recryst from H_2O and dried in air to give the $1.5.H_2O$ and has **m** 80°. Anhydrous surfactant is obtained by drying at 78° over P_2O_5. [Heilfrich and Türk *Chem Ber* **89** 2215 *1856*.]

Phenyl-β-D-galactopyranoside *[2818-58-8]* **M 256.3, m 153-54°, 146-148°, 155-156°(dried at 105°), $[\alpha]_D^{20}$ -42° (c 1, H_2O).** Recrystd from H_2O as $0.5H_2O$. [Conchie and Hay *Biochem J* **73** 327 *1959*; IR: Whistler and House *Analyt Chem* 25 1463 *1953*.] It is an acceptor substrate for fucosyltransferase [Chester et al. *Eur J Biochem* **69** 583 *1976*].

Phenyl-β-D-glucopyranoside *[1464-44-4]* **M 256.3, m 174-175° 174-176°, 176°, $[\alpha]_D^{20}$ -72.2° (c 1 for dihydrate, H_2O).** Recrystd from H_2O as $2H_2O$ and can be dried *in vacuo* at 100°/P_2O_5. Dry preparation has $[\alpha]_D^{25}$ -70.7° (c 2, H_2O). [Robertson and Waters *J Chem Soc* 2729 *1930*; IR: Bunton et al. *J Chem Soc* 4419 *1955*; Takahashi *Yakugaku Zasshi (J Pharm Soc Japan)* **74** 7436 *1954*; Whixtler and House *Anal Chem* **25** 1463; UV: Lewis *J Am Chem Soc* **57** 898 *1935*.] It is a substrate for β–D-glucosidase [deBryne *Eur J Biochem* **102** 257 *1979*].

Phenylmercuric acetate (PhHgOAc) *[62-38-4]* **M 336.7, m 148-151°, 149°, 151.8-152.8°.** Small colourless lustrous prisms from EtOH. Its solubility in H_2O is 0.17% but it is more soluble in EtOH, Me_2CO and $^*C_6H_6$. [Maynard *J Am Chem Soc* **46** 1510 *1925*; Coleman et al. *J Am Chem Soc* **59** 2703 *1937*; *J Am Pharm Assoc* **25** 752 *1936*.] See PhHgOH and $PhHgNO_3$.PhHgOH on p. 449 in Chapter 5.

Phenylmethanesulfonyl fluoride (PMSF) *[329-98-6]* **M 174.2, m 90-91°, 92-93°.** Purified by recrystn from $^*C_6H_6$, pet ether or $CHCl_3$-pet ether. [Davies and Dick *J Chem Soc* 483 *1932*; cf Tullock and Coffman *J Org Chem* **23** 2016 *1960*.] It is a general protease inhibitor (specific for trypsin and chymotrypsin) and is a good substitute for diisopropylphosphoro floridate [Fahrney and Gould *J Am Chem Soc* **85** 997 *1963*].

Phosphatase alkaline (alkaline phosphatase) *[9001-78-9]* **M_r ~40,000 (bovine liver), ~140,000 (bovine intestinal mucosa), 80,000 (*E.coli*) [EC 3.1.3.1].** The *E.coli* supernatant in sucrose (20%, 33mM) in Tris-HCl pH 8.0 was purified through a DEAE-cellulose column and recrystallised. To the column eluates in 0.125M NaCl is added $MgCl_2$ (to 0.01M) and brought to 50% saturation in $(NH_4)_2SO_4$ by adding the solid (0.20g/mL). The mixture is centrifuged to remove bubbles and is adjusted to pH 8.0 (with 2N NaOH). Saturated $(NH_4)_2SO_4$ at pH 8.0 is added dropwise until the soln becomes faintly turbid (~61% saturation). It is set aside at room temp for 1h (turbidity will increase). The mixture is placed in an ice bath for several minutes when turbidity disappears and a clear soln is obtained. It is then placed in a large ice bath at 0° (~5L) and allowed to warm slowly to room temperature in a dark room whereby crystals are formed appearing as a silky sheen. The crystals are collected by centrifugation at 25° if necessary. The crystalline solns are stable at room temperature for many months. They can be stored at 0°, but are not stable when frozen. Cysteine at 10^{-3}M and thioglycolic acid at 10^{-4}M are inhibitory. Inhibition is reversed on addition of Zn^{2+} ions. Many organic phosphates are good substrates for this phosphatase. [Molamy and Horecker *Methods Enzymol* **9** 639 *1966*; Torriani et al. *Methods Enzymol* **12b** 212 *1968*; Engstrom *Biochim Biophys Acta* **92** 71 *1964*.]
Alkaline phosphatase from rat *osteosarcoma* has been purified by acetone pptn, followed by chromatography on DEAE-cellulose, Sephacryl S-200, and hydroxylapatite. [Nair et al. *Arch Biochem Biophys* **254** 18 *1987*.]

3-sn-Phosphatidylethanolamine (L-α-cephalin, from Soya bean) *[39382-08-6]* **M_r ~600-800, amorphous, $pK_{Est(1)}$~ 5.8 (PO_4^-), $pK_{Est(2)}$~ 10.5 (NH_2).** Purified by dissolving in EtOH, adding $Pb(OAc)_2.3H_3O$ (30g in 100mL H_2O) until excess Pb^{2+} is present. Filter off the solid. Pass CO_2 gas through

the soln until pptn of $PbCO_3$ ceases. Filter the solid off and evaporate (while bubbling CO_2) under vacuum. An equal volume of H_2O is added to the residual oil extracted with hexane. The hexane extract is washed with H_2O until the aqueous phase is free from Pb [test with dithizone (2 mg in 100 mL CCl_4 ; Feigel *Spot Tests* Vol I, Elsevier p. 10 *1954*]. The hexane is dried (Na_2SO_4), filtered and evaporated to give a yellow waxy solid which should be dried to constant weight *in vacuo*. It is practically insoluble in H_2O and Me_2CO, but freely soluble in $CHCl_3$ (5%) and Et_2O, and slightly soluble in EtOH. [Schofield and Dutton *Biochem Prep* **5** 5 *1957*.]

***O*-Phosphocolamine 2-aminoethyl dihydrogen phosphate)** *[1071-23-4]* **M 141.1, m 237-240°, 242.3°, 234.5-244.5°, 244-245°(capillary), pK_1^{20} <1.5 (PO_4H_2), pK_2^{20} 5.77 (PO_4H^-), pK_3^{20} 10.26 (NH^+).** Purified by recrystn from aqueous EtOH as a hydrate (**m** 140-141°). Its solubility in H_2O is 17% and 0.003% in MeOH or EtOH at 22°. [Fölisch and Österberg *J Biol Chem* **234** 2298 *1959*; Baer aand Staucer *Can J Chem* **34** 434 *1956*; Christensen *J Biol Chem* **135** 399 *1940*.] It is a potent inhibitor of ornithine decarboxylase [Gilad and Gilad *Biochem Biophys Res Commun* 122 277 *1984*].

Phosphoenolpyruvic acid monopotassium salt (KPEP) *[4265-07-0]* **M 206.1, pK_1^{25} 3.4 (CO_2), pK_2^{25} 6.35 (PO_4H^-) (for free acid).** It is purified *via* the monocyclohexylamine salt (see next entry). The salt (534mg) in H_2O (10mL) is added to Dowex 50Wx4 H^+ form (200-400 mesh, 2mL, H_2O washed) and stirred gently for 30min and filtered. The resin is washed with H_2O (6mL) and the combined solns are adjusted to pH 7.4 with 3N KOH (~1.4mL) and the volume adjusted to 18.4mL with H_2O to give a soln of 0.1M KPEP which can be lyophilised to a pure powder and is very good for enzyme work. It has been recrystd from MeOH-Et_2O. [Clark and Kirby *Biochem Prep* **11** 103 *1966*; Wold and Ballou *J Biol Chem* **227** 301 *1957*; Cherbuliez and Rabinowitz *Helv Chim Acta* **39** 1461 *1956*.]
The triNa salt *[5541-93-5]* **M 360.0,** is purified as follows: the salt (1g) is dissolved in MeOH (40mL) and dry Et_2O is added in excess. The white crystals are collected and dried over P_2O_5 at 20°. [*Chem Ber* **92** 952 *1959*.]

Phosphoenolpyruvic acid tris(cyclohexylamine) salt *[35556-70-8]* **M 465.6, m 155-180°(dec).** Recrystd from aqueous Me_2CO and dried in a vacuum. At 4° it is stable for >2 years and has IR at 1721cm^{-1} (C=O). [Wold and Ballou *J Biol Chem* **227** 301 *1957*; Clark and Kirby *Biochem Prep* **11** 103 *1966* for the monocyclohexylamine salt.]

D-3-Phosphoglyceric acid disodium salt (D-glycerate 3-phosphate di-Na salt) *[80731-10-8]* **M 230.0, $[\alpha]_D^{25}$ +7.7° (c 5, H_2O), -735° (in aq NH_4^+ molybdate), $pK_{Est(1)}$~1.0 (PO_4H_2), $pK_{Est(2)}$~ 6.66 (PO_4H^-) (for free acid).** Best purified by conversion to the Ba salt by pptn with $BaCl_2$ which is recrystd three times before conversion to the sodium salt. The Ba salt (9.5g) is shaken with 200mL of a 1:1 slurry of Dowex 50 (Na^+ form) for 2h. The mixture is filtered and the resin washed with H_2O (2 x 25mL). The combined filtrates (150mL) are adjusted to pH 7.0 and concentrated *in vacuo* to 30-40mL and filtered if not clear. Absolute EtOH is added to make 100mL and then *n*-hexane is added whereby a white solid and/or a second phase separates. When set aside at room temperature complete pptn of the Na salt as a solid occurs. The salt is removed by centrifugation, washed with Me_2CO, dried in air then in an oven at 55° to give a stable powder (4.5g). It did not lose weight when dried further over P_2O_5 at 78°/8h. The high rotation in the presence of $(NH_4)_6Mo_7O_{24}$ is not very sensitive to the concentration of molybdate or pH as it did not alter appreciably in 1/3 volume between 2.5 to 25% (w/v) of molybdate or at pH values ranging between 4 and 7. [Cowgill *Biochim Biophys Acta* **16** 613 *1955*; Embdan, Deuticke and Kraft *Hoppe Seyler's Z Physiol Chem* **230** 20 *1934*.]

Phospholipids. For the removal of ionic contaminants from raw zwitterionic phospholipids, most lipids were purified twice by mixed-bed ionic exchange (Amberlite AB-2) of methanolic solutions. (About 1g of lipid in 10mL of MeOH). With both runs the first 1mL of the eluate was discarded. The main fraction of the solution was evaporated at 40°C under dry N_2 and recryst three times from *n*-pentane. The resulting white powder was dried for about 4h at 50° under reduced pressure and stored at 3°. Some samples were purified by mixed-bed ion exchange of aqueous suspensions of the crystal/liquid crystal phase. [Kaatze et al. *J Phys Chem* **89** 2565 *1985*.]

Phosphoproteins (various). Purified by adsorbing onto an iminodiacetic acid substituted agarose column to which was bound ferric ions. This chelate complex acted as a selective immobilised metal affinity adsorbent for phosphoproteins. [Muszyfiska et al. *Biochemistry* **25** 6850 *1986.*]

5'-Phosphoribosyl pyrophosphate synthetase (from human erythrocytes, or pigeon or chicken liver) *[9015-83-2]* **M_r 60,000, [EC 2.7.6.1].** Purified 5100-fold by elution from DEAE-cellulose, fractionation with ammonium sulfate, filtration on Sepharose 4B and ultrafiltration. [Fox and Kelley *J Biol Chem* **246** 5739 *1971*; Flaks *Methods Enzymol* **6** 158 *1963*; Kornberg et al. *J Biol Chem* **15** 389 *1955.*]

***O*-Phospho-L-serine** *[407-41-0]* **M 185.1, m 175-176°, $[\alpha]_D^{20}$ +4.3° (c 3.2, H_2O), +16.2° (c 3.2, 2N HCl), pK_1^{25}<1 (PO_4H_2), pK_2^{25} 2.08 (CO_2H), pK_3^{25} 5.65 (PO_4H^-), pK_4^{25} 9.74 (NH_3^+).** Recrystd by dissolving 10g in H_2O (150mL) at 25°, stirring for up to 20min. Undissolved material is filtered off (Büchner) and 95% EtOH (85mL) is added dropwise during 4min, and set aside at 25° for 3h then at 3° overnight. The crystals are washed with 95% EtOH (100mL) then dry Et_2O (50mL) and dried in a vacuum (yield 6.5g). A further quantity (1.5mg) can be obtained by keeping the mother liquors and washings at -10° for 1 week. The *DL-isomer* has **m** 167-170°(dec) after recrystn from H_2O + EtOH or MeOH. [Neuhaus and Korkes *Biochem Prep* **6** 75 *1958*; Neuhaus and Byrne *J Biol Chem* **234** 113 *1959*; IR: Fölsch and Mellander *Acta Chem Scand* **11** 1232 *1957.*]

***O*-Phospho-L-threonine (L-threonine-*O*-phosphate)** *[1114-81-4]* **M 199.1, m 194°(dec), $[\alpha]_D^{24}$ -7.37° (c 2.8, H_2O) (pK as above).** Dissolve in the minimum volume of H_2O, add charcoal, stir for a few min, filter and apply onto a Dowex 50W (H^+ form) then elute with 2N HCl. Evaporate the eluates under reduced pressure whereby the desired fraction produced crystals of the phosphate which can be recrystd from H_2O-MeOH mixtures and the crystals are then dried *in vacuo* over P_2O_5 at ~80°. [de Verdier *Acta Chem Scand* **7** 196 *1953.*]

***O*-Phospho-L-tyrosine (L-tyrosine-*O*-phosphate)** *[21820-51-9]* **M 261.2, m 225°, 227°, 253°, $[\alpha]_D^{20}$ -5.5° (c 1, H_2O), -9.2° (c 1, 2N HCl), $pK_{Est(1)}$~ 1.6 (PO_4H_2), $pK_{Est(2)}$~ 2.02 (CO_2H), $pK_{Est(3)}$~ 5.65 (PO_4H^-), $pK_{Est(4)}$ 9.2 (NH_3^+).** Purified by recrystn from H_2O or H_2O + EtOH. [Levene and Schormüller *J Biol Chem* **100** 583 *1933*; Posternak and Graff *Helv Chim Acta* **28** 1258 *1945.*]

Phytol (*d*-3,7*R*,11*R*,15-tetramethylhexadec-2-en-1-ol) *[150-86-7]* **M 296.5, b 145°/0.03mm, 150-151°/0.06mm, 202-204°/10mm, d_4^{25} 0.8497, n_D^{25} 1.437, $[\alpha]_D^{22}$+0.06° (neat).** Purified by distn under high vacuum. It is almost insoluble in H_2O but soluble in most organic solvents. It has UV λ_{max} at 212nm (log ε 3.04) in EtOH and IR ν at 3300 and 1670cm^{-1}. [Demole and Lederer *Bull Soc Chim Fr* 1128 *1958*; Burrell *J Chem Soc (C)* 2144 *1966*; Bader *Helv Chim Acta* **34** 1632 *1951.*]

D-Pipecolinic acid (*R*-piperidine-2-carboxylic acid) *[1723-00-8]* **M 129.2, m 264°(dec), 267°(dec), ~280°(dec), $[\alpha]_D^{19}$+26.2° (c 2, H_2O), $[\alpha]_D^{25}$+35.7° (H_2O), pK_1^{20} 2.29 (CO_2H), pK_2^{20} 10.77 (NH^+).** Recrystallises as platelets from EtOH and is soluble in H_2O. The *hydrochloride* has **m** 256-257°(dec) from H_2O and $[\alpha]_D^{25}$ +10.8° (c 2, H_2O). [Lukés et al. *Collect Czech Chem Commun* **22** 286 *1957*; Bayerman *Recl Trav Chim Pays-Bas* **78** 134 *1959*; Asher et al. *Tetrahedron Lett* **22** 141 *1981.*]

L-Pipecolinic acid (*S*-piperidine-2-carboxylic acid) *[3105-95-1]* **M 129.2, m 268°(dec), 271°(dec), ~280°(dec), $[\alpha]_D^{20}$ -26° (c 4, H_2O), $[\alpha]_D^{25}$ -34.9° (H_2O).** Recrystd from aqueous EtOH and sublimes as needles in a vacuum. It is sparingly soluble in absolute EtOH, Me_2CO and $CHCl_3$ but insoluble in Et_2O. The *hydrochloride* has **m** 258-259°(dec, from MeOH) and $[\alpha]_D^{25}$ -10.8° (c 10, H_2O). [Fujii and Myoshi *Bull Chem Soc Jpn* **48** 1241 *1975.*]

Piperidine-4-carboxylic acid (isonipecotic acid) *[498-94-2]* **M 129.2, m 336°(dec, darkens at ~300°), $pK_{Est(1)}$~ 4.3 (CO_2H), $pK_{Est(2)}$~ 10.6 (NH^+).** Recrystallises from H_2O or EtOH as needles. The *hydrochloride* recrystallises from H_2O or aqueous HCl and has **m** 293°dec (298°dec, 300°dec). [Wibaut *Recl Trav Chim Pays-Bas* **63** *141* 1944; IR: Zacharius et al. *J Am Chem Soc* **76** 2908 *1954.*]

Pituitary Growth Factor (from human pituitary gland) *[336096-71-0].* Purified by heparin and copper affinity chromatography, followed by chromatography on carboxymethyl cellulose (Whatman 52). [Rowe et al. *Biochemistry* **25** 6421 *1986.*]

Plasmids. These are circular lengths of DNA which invade bacteria or other cells e.g. insect cells, yeast cells, and have sequences which are necessary for their replication using enzymes and other ingredients, e.g. nucleotides, present in the cells. They contain engineered, or already have, genes which produce enzymes that provide the cells with specific antibiotic resistance and are thus useful for selecting bacteria containing specific plasmids. Plasmids have been extremely useful in molecular biology since they can be very easily identified (from their size or the sizes of the DNA fragments derived from their restriction enzyme digests) and can be readily engineered *in vitro* (outside the cells). Genes coding for specific enzymes or other functional proteins can be inserted into these plasmids which have DNA sequences that allow the expression of large quantities of bacteria or non-bacterial (e.g. human) proteins. They have also been engineered in such a way as to produce 'fusion proteins' (in which the desired protein is fused with a specific "reporter, marker or carrier protein" which will facilitate the isolation of the desired protein (e.g. by binding strongly to a nickel support) and then the desired protein can be cleaved from the eluted fusion protein and obtained in very pure form. A large number of plasmids with a variety of sequences for specific purposes are commercially available in very pure form. They can be used to infect cells and can be isolated and purified from cell extracts in large amounts using a number of available procedures. These procedures generally involve lysis of the cells (e.g. with alkaline sodium dodecylsulfate, SDS), separation from nuclear DNA, precipitation of plasmid DNA from the cell debris, adsorbing it on columns which specifically bind DNA, and then eluting the DNA from the column (e.g. with specific Tris buffers as recommended by the suppliers) and precipitating it (e.g. with Tris buffer in 70% EtOH at -70°C) The purity is checked in agarose gel (containing ethidium bromide to visualise the DNA) by electrophoresis. A large number of plasmids are now commercially available (see Clontech GmbH, http://www.clontech.com; Invitrogen http://www.invitrogen.com, among other suppliers) used as vectors for bacterial, mammalian, yeast and baculovirus expression.

Podophylotoxin *[518-28-5]* **M 414,4, m 181-181°, 183-184°, 188-189°, $[\alpha]_D^{20}$ -132° (c 1, $CHCl_3$).** Recrystallises form $^*C_6H_6$ (with $0.5C_6H_6$), EtOH-$^*C_6H_6$, aqueous EtOH (with $1\text{-}1.5H_2O$, **m** 114-115°) and CH_2Cl_2-pentane. When dried at 100°/10 mm it has **m** 183-184°. [UV: Stoll et al. *Helv Chim Acta* **37** 1747 *1954*; IR: Schecler et al. *J Org Chem* **21** 288 *1956.*] Inhibitor of microtubule assembly [Prasad et al. *Biochemistry* **25** 739 *1986*].

Polyethylene glycol *[25322-68-3]* **M_r various, from ~200 to ~35,000.** May be contaminated with aldehydes and peroxides. Methods are available for removing interfering species. [Ray and Purathingal *Anal Biochem* **146** 307 *1985.*]

Polypeptides. These are a string of α-amino acids usually with the natural *S*(L) [L-cysteine is an exception and has the *R* absolute configuration] or sometimes "unnatural" *R*(D) configuration at the α-carbon atom. They generally have less than ~100 amino acid residues. They can be naturally occurring or, because of their small size, can be synthesised chemically from the desired amino acids. Their properties can be very similar to those of small proteins. Many are commercially available, can be custom made commercially or locally with a peptide synthesiser. They are purified by HPLC and can be used without further purification. Their purity can be checked as described under proteins.

Porphobilinogen (5-amino-4-carboxymethyl-1*H*-pyrrole-3-propionic acid) *[487-90-1]* **M 226.2, m 172-175°(dec), 175-180°(dec, darkening at 120-130°), pK_1 3.70 ($4\text{-}CH_2CO_2H$), pK_2 4.95 ($3\text{-}CH_2CH_2CO_2H$), pK_3 10.1 (NH^+).** Recrystallises as the monohydrate (pink crystals) from dil NH_4 OAc solns of pH 4, and is dried *in vacuo*. The *hydrochloride monohydrate* has **m** 165-170°(dec) (from dilute HCl). [Jackson and MacDonald *Can J Chem* **35** 715 *1957*, Westall *Nature* **170** 614 *1952*; Bogarad *J Am Chem Soc* **75** 3610 *1953.*]

Porphyrin a (from ox heart) *[5162-02-1]* **M 799.0, m dec on heating.** Purified on a cellulose powder column followed by extraction with 17% HCl and fractionation with HCl. [Morell et al. *Biochem J* **78**

793 *1961*.] Recrystd from $CHCl_3$/Pet ether or Et_2O/*C_6H_6 [detailed UV-VIS and NMR date: Caughey et al. *J Biol Chem* **250** 7602 *1975*; Lemberg *Adv Enzymol* **23** 265 *1961*].

Prazosin hydrochloride (2[4-{(2-furoyl)piperazin-1-yl}4-amino-6,7-dimethoxyquinazoline hydrochloride) *[19237-84-4]* **M 419.9, m 278-280°, 280-282°, pK 6.5.** It is recrystd by dissolving in hot MeOH adding a small volume of MeOH-HCl (dry MeOH saturated with dry HCl gas) followed by dry Et_2O until crystn is complete. Dry *in vacuo* over solid KOH till odour of HCl is absent. It has been recrystd from hot H_2O, the crystals were washed with H_2O, and the H_2O was removed azeotropically with CH_2Cl_2, and dried in a vacuum. [NMR and IR: Honkanen et al. *J Heterocycl Chem* **17** 797 *1980*; *cf* Armarego and Reece *Aust J Chem* **34** 1561 *1981*.] It is an antihypertensive drug and is an α_1-adrenergic antagonist [Brosman et al. *Proc Natl Acad Sci USA* **82** 5915 *1985*].

Prednisolone acetate (21-acetoxypregna-1,4-diene-11β-17α-diol-3,20-dione) *[52-21-1]* **M 402.5, m 237-239°, 240-242°, 240-243°, 244°, $[\alpha]_D^{20}$ +116° (c 1, dioxane).** Recrystd from EtOH, Me_2CO, Me_2CO-hexane, and has UV λ_{max} at 243nm in EtOH. [Joly et al. *Bull Soc Chim Fr* 366 *1958*; Herzog et al. *J Am Chem Soc* **77** 4781 *1955*.]

Primaquine diphosphate (*RS*- 8-[4-amino-1-methylbutylamino]-6-methoxyquinoline diphosphate) *[63-45-6]* **M 455.4, m 197-198°, 204-206°, $pK_{Est(1)}$~ 3.38 (ring N^+), $pK_{Est(2)}$~ 10.8 NH_3^+).** It forms yellow crystals from 90% aq EtOH and is moderately soluble in H_2O. The *oxalate salt* has **m** 182.5-185° (from 80% aq EtOH) and the *free base* is a viscous liquid **b** 165-170°/0.002mm, 175-177°/2mm. [Elderfield et al. *J Am Chem Soc* **68** 1526 *1964*; **77** 4817 *1955*.]

Procaine hydrochloride (Novocain, 2-diethylaminoethyl-4-aminobenzoate) *[51-05-8]* **M 272.8, m 153-156°, 154-156°, 156°, $pK_{Est(1)}$~ 2.52 (NH_2^+) pK_2^{20} 9.0 (Et_2N^+).** Recrystd from aqueous EtOH. It has solubility at 25° in H_2O (86.3%), EtOH (2.6%) and Me_2CO (1%), it is slightly soluble in $CHCl_3$ but is almost insoluble in Et_2O. The anhydrous *free base* is recrystd from ligroin or Et_2O and has **m** 61°. [Einhorn *Justus Liebigs Ann Chem* **371** 125 *1909*; IR: Szymanski and Panzica *J Amer Pharm Assoc* **47** 443 *1958*.]

L-Propargylglycine (*S*-2-aminopent-4-ynoic acid) *[23235-01-0]* **M 113.1, m 230°(dec starting at 210°), $[\alpha]_D^{20}$ -35° (c 1, H_2O), -4° (c 5, 5N HCl), $pK_{Est(1)}$~ 2.3 (CO_2H), $pK_{Est(2)}$~ 9.8 (NH_2).** The acid crystallises readily when ~4g in 50mL H_2O is treated with abs EtOH at 4°/ 3hrs, and is collected washed with cold abs EtOH and Et_2O and dried in vac. Also recrystallises from aqueous Me_2CO, R_F on SiO_2 TLC plates with *n*-BuOH-H_2O-AcOH (4:1:1) is 0.26. The *racemate* has **m** 238-240°. [Leukart et al. *Helv Chim Acta* **59** 2181 *1976*; Eberle and Zeller *Helv Chim Acta* **68** 1880 *1985*; Jansen et al. *Recl Trav Chim Pays-Bas* **88** 819 *1969*.] It is a suicide inhibitor of γ-cystathionase and other enzymes [Washtier and Abeles *Biochemistry* **16** 2485 *1977*; Shinozuka et al. *Eur J Biochem* **124** 377 *1982*].

Propidium iodide (3,8-diamino-5-(3-diethylaminopropyl)-6-phenylphenantridinium iodide methiodide) *[25535-16-4]* **M 668.4, m 210-230°(dec), $pK_{Est(1)}$~ 4 (aniline NH_2), $pK_{Est(2)}$~ 8.5 (EtN_2).** Recrystd as red crystals from H_2O containing a little KI. It fluoresces strongly with nucleic acids. [Eatkins *J Chem Soc* 3059 *1952*.] **TOXIC.**

***R*-Propranalol hydrochloride (*R*-1-isopropylamino-3-(1-naphthyloxy)-2-propanol HCl)** *[13071-11-9]* **M 295.8, m 192°, 193-195°, $[\alpha]_D^{20}$ -25° (c 1, EtOH), pK^{20} 9.5 (for free base).** Recryst from *n*-PrOH or Me_2CO. It is soluble in H_2O and EtOH but is insoluble in Et_2O, *C_6H_6 or EtOAc. The *racemate* has **m** 163-164°, and the *free base* recryst from cyclohexane has **m** 96°. [Howe and Shanks *Nature* **210** 1336 *1966*.] The *S*-isomer (below) is the physiologically active isomer.

***S*-Propranalol hydrochloride (*S*-1-isopropylamino-3-(1-naphthyloxy)-2-propanol HCl)** *[4199-10-4]* **M 295.8, m 192°, 193-195°, $[\alpha]_D^{20}$ +25° (c 1, EtOH) pK^{20} 9.5.** See preceding entry for physical properties. The is the active isomer which blocks isoprenaline tachycardia and is a β-adrenergic blocker. [Leclerc et al. *Trends Pharmacol Sci* **2** 18 *1981*; Howe and Shanks *Nature* **210** 1336 *1966*.]

Protamine kinase (from rainbow trout testes) *[37278-10-7]* **[EC 2.7.1.70].** Partial purification by hydoxylapatite chromatography followed by biospecific chromatography on nucleotide coupled Sepharose 4B (the nucleotide was 8-(6-aminohexyl)amine coupled cyclic-AMP). [Jergil et al. *Biochem J* **139** 441 *1974.*]

Protamine sulfate (from herring sperm) *[9007-31-2]* $[\alpha]_D^{22}$ **-85.5° (satd H_2O), pK 7.4-8.0.** A strongly basic protein (white powder, see pK) used to ppte nucleic acids from crude protein extracts. It dissolved to the extent of 1.25% in H_2O. It is freely soluble in hot H_2O but separates as an oil on cooling. It has been purified by chromatography on an IRA-400 ion-exchange resin in the SO_4^{2-} form and washed with dilute H_2SO_4. Eluates are freeze-dried under high vacuum below 20°. This method is used to convert proteamine and protamine hydrochloride to the sulfate. [UV: Rasmussen *Hoppe Seyler's Z Physiol Chem* **224** 97 *1934*; Ando and Sawada *J Biochem (Tokyo)* **49** 252 *1961*; Felix and Hashimoto *Hoppe Seyler's Z Physiol Chem* **330** 205 *1963*]

Protease nexin (From cultured human fibroblasts) *[148263-58-5].* Purified by affinity binding of protease nexin to dextran sulfate-Sepharose. [Farrell et al. *Biochem J* **237** 707 *1986.*]

Proteins. These are usually naturally occurring (or deliberately synthesised in microorganisms, e.g. bacteria, insect cells, or animal tissues), and are composed of a large number of α-*S* (L)amino acids residues (except for L-cysteine which has the *R* absolute configuration), selected from the 20 or so natural amino acids, in specific sequences and in which the α–amino group forms an amide (peptide) bond with the α–carboxyl group of the neighboring amino acid. The number of residues are ususally upwards of 100. Proteins with less than 100 amino acids are better referred to as **polypeptides**. Aqueous soluble proteins generally fold into ball-like structures mainly with hydrophilic residues on the outside of the "balls" and hydrophobic residues on the inside. Proteins can exist singly or can for dimers, trimers, tetramers etc, consisting of similar or different protein subunits. They are produced by cells for a large variety of functions, e.g. enzymology, reaction mediation as in regulation of DNA synthesis or chaperonins for aiding protein folding, formation of pores in membranes for transport of ions or organic molecules, or for intra or inter cellular signalling etc. The purity of proteins can be checked in denaturing (SDS, sodium dodecylsulfate) or non-denaturing polyacrylamide gels using electrophoresis (PAGE), and staining appropriately (e.g. with Coommassie Blue, followed by silver staining for higher sensitivity). If the protein is partly impure then it should be purified further according to the specific literature procedures for the individual protein (see specific proteins in the *Methods Enzymol* , Wiley series).

Proteoglycans (from cultured human muscle cells). Separated by ion-exchange HPLC using a Bio-gel TSK-DEAE 5-PW analytical column. [Harper et al. *Anal Biochem* **159** 150 *1986.*]

Prothrombin (Factor II, from equine blood plasma) *[9001-26-7]* M_r **72,000.** Purified by two absorptions on a barium citrate adsorbent, followed by decomposition of the adsorbents with a weak carboxylic cation-exchanger (Amberlite IRF-97), isoelectric pptn (pH 4.7-4.9) and further purification by chromatography on Sephadex G-200 or IRC-50. Finally recrystd from a 1% soln adjusted to pH 6.0-7.0 and partial lyophilisation to *ca* 1/5 to 1/10th vol and set aside at 2-5° to crystallise. Occasionally seeding is required. [Miller *Biochem Prep* **13** 49 *1971.*]

Protoporphyrin IX (3,18-divinyl-2,7,13,17-tetramethylporphine-8,12-dipropionic acid, ooporphyrin) *[553-12-8]* **M 562.7, pK_{Est} ~ 4.8.** Purified by dissolving (4g) in 98-100% HCOOH (85mL), diluting with dry Et_2O (700mL) and keeping at 0° overnight. The ppte is collected and washed with Et_2O then H_2O and dried in a vacuum at 50° over P_2O_5. It has been recrystd from aqueous pyridine and from Et_2O as monoclinic, brownish-yellow prisms. UV λ_{max} values in 25% HCl are 557.2, 582.2 and 602.4nm. It is freely soluble in ethanolic HCl, AcOH, $CHCl_3$, and Et_2O containing AcOH. It forms sparingly soluble diNa and diK salts. [Ramsey *Biochem Prep* **3** 39 *1953*; UV: Holden *Aust J. Exptl Biol and Med Sci* **15** 412 *1937*; Garnick *J Biol Chem* **175** 333 *1948*; IR: Falk and Willis *Aust J Sci Res* [A] **4** 579 *1951.*]
The **Dimethyl ester** *[5522-66-7]* **M 590.7, m 228-230°**, is prepared by dissolving (0.4g) in $CHCl_3$ (33mL) by boiling for a few min, then diluting with boiling MeOH (100mL) and refrigerating for 2 days. The crystals are collected, washed with $CHCl_3$-MeOH (1:9) and dried at 50° in a vacuum (yield 0.3g). UV has λ_{max} 631, 576, 541, 506 and 407nm in $CHCl_3$ and 601, 556 and 406nm in 25% HCl. [Ramsey *Biochem Prep* **3** 39 *1953.*]

Prymnesin (toxic protein from phytoflagellate *Pyrymnesium parvum*) *[11025-94-8].* Purified by column chromatography, differential soln and pptn in solvent mixtures and differential partition between diphasic mixtures. The product has at least 6 components as observed by TLC. [Ulitzur and Shilo *Biochim Biophys Acta* **301** 350 *1970.*]

Pterin-6-carboxylic acid (2-amino-4-oxo-3,4-dihydropteridine-6-carboxylic acid) *[948-60-7]* **M 207.2, m >360°, pK_1^{20} 1.43, pK_2^{20} 2.88, pK_3^{20} 7.72.** Yellow crystals by repeated dissolution in aqueous NaOH and adding aqueous HCl. It has UV with λ_{max} at 235, 260 and 265nm (ε 11000, 10500 and 9000) in 0.1N HCl and 263 and 365nm (ε 20500 and 9000) in 0.1N NaOH. [UV: Pfleiderer et al. *Justus Liebigs Ann Chem* **741** 64 *1970*; Stockstad et al. *J Am Chem Soc* **70** 5 *1948*; Fluorescence: Kavanagh and Goodwin *Arch Biochem* **20** 315 *1949.*]

Purine-9-β-ribifuranoside (Nebularin) *[550-33-4]* **M 252.2, m 178-180°, 181-182°, $[\alpha]_D^{25}$ -48.6° (c 1, H_2O), -22° (c 0.8, 0.1N HCl) and -61° (c 0.8, 0.1N NaOH), pK 2.05.** Recrystd from butanone + MeOH or EtOH and forms a MeOH photo-adduct. It is a strong inhibitor of adenosine deaminase [EC 3.5.4.4]. [Nair and Weichert *Bioorg Chem* **9** 423 *1980*; Löfgren et al. *Acta Chem Scand* **7** 225 *1953*; UV: Brown and Weliky *J Biol Chem* **204** 1019 *1953.*]

Puromycin dihydrochloride (*O*-methyl-l-tyrosine[N^6,N^6-dimethylaminoadenosin-3'-yl-amide]) *[58-58-2]* **M 616.5, m 174°, $[\alpha]_D^{25}$ -11° (free base in EtOH), pK_1 6.8, pK_2 7.2.** Purified by recrystn from H_2O. The *free base* has **m** 175.5-177° (172-173°) (from H_2O). The *sulfate* has **m** 180-187° dec (from H_2O), and the *picrate monohydrate* has **m** 146-149° (from H_2O). [Baker et al. *J Am Chem Soc* **77** 1 *1955*; Fryth et al. *J Am Chem Soc* **80** 3736 *1958.*] It is an inhibitor of aminopeptidase and terminates protein synthesis [Reboud et al. *Biochemistry* **20** 5281 *1981*].

Pyridoxal hydrochloride *[65-22-5]* **M 203.6, m 176-180°(dec), pK_1^{20} 4.23 (3-OH), pK_2^{20} 8.7 (Pyridinium$^+$), pK_3^{20} 13.04 (CH_2OH?).** Dissolve in water and adjust the pH to 6 with NaOH. Set aside overnight to crystallise. The crystals are washed with cold water, dried in a vacuum desiccator over P_2O_5 and stored in a brown bottle at room temperature. [Fleck and Alberty *J Phys Chem* **66** 1678 *1962.*]

Pyridoxal-5'-phosphate monohydrate (PLP, codecarboxylase) *[54-47-7]* **M 265.2, pK_1^{25} <2.5 (PO_4), pK_2^{25} 4.14 (3-OH), pK_3^{25} 6.20 (PO_4), pK_4^{25} 8.69 (pyridinium$^+$).** It has been purified by dissolving 2g in H_2O (10-15mL, in a dialysis bag a third full) and dialysing with gentle stirring against 1L of H_2O (+ two drops of toluene) for 15h in a cold room. The dialysate is evaporated to 80-100mL then lyophilised. Lemon yellow microscopic needles of the monohydrate remain when all the ice crystals have been removed. The purity is checked by paper chromatography (in EtOH or *n*-PrOH-NH_3) and the spot(s) visualised under UV light after reaction with *p*-phenylene diamine, NH_3 and molybdate. Solutions stored in a freezer are 2-3% hydrolysed in 3-weeks. At 25°, only 4-6% hydrolysis occurs even in N NaOH or HCl, and 2% is hydrolysed at 37° in 1 day - but is complete at 100° in 4h. Best stored as dry solid at -20°. In aqueous acid the solution is colourless but is yellow in alkaline solutions. It has UV λ_{max} at 305nm (ε 1100) and 380nm (ε 6550) in 0.1 N NaOH; 330nm (ε 2450) and 388nm (ε 4900) in 0.05M phosphate buffer pH 7.0 and 295nm (ε 6700) in 0.1N HCl. [Peterson et al. *Biochemical Preparations* **3** 34, 119 *1953.*] The *oxime* dec at 229-230° and is practically insoluble in H_2O, EtOH and Et_2O. The *O-methyloxime* decomposes at 212-213°. [Heyl et al *J Am Chem Soc* **73** 3430 *1951.*] It has also been purified by column chromatography through Amberlite IRC-50 (H^+) [Peterson and Sober *J Am Chem Soc* **76** 169 *1954*].

Pyridoxamine hydrochloride *[5103-96-8, 524-36-7 (free base)]* **M 241.2, m 226-227°(dec), pK_1^{25} 3.54 (3-OH), pK_2^{25} 8.21 (ring N$^+$), pK_3^{25} 10.63 (NH_2).** Crystd from hot MeOH. The *free base* crysts from EtOH, has **m** 193-193.5° [Harris et al. *J Biol Chem* **154** 315 *1944*, *J Am Chem Soc* **66** 2088 *1944*].

Pyridoxine hydrochloride see **Vitamin B_6.**

Pyruvate kinase isoenzymes (from *Salmonella typhimurium*) *[9001-59-6]* **M_r 64,000, [EC 2.7.1.40], amorphous.** Purified by $(NH_4)_2SO_4$ fractionation and gel filtration, ion-exchange and affinity chromatography. [Garcia-Olalla and Garrido-Pertierra *Biochem J* **241** 573 *1987.*]

Quinacrine **[Atebrine, 3-chloro-9(4-diethylamino-1-methyl)butylamino-7-methoxy)acridine] dihydrochloride.** *[69-05-6]* **M 472.9, m 248-250°(dec), pK_1^{30} -6.49 (aq H_2SO_4), pK_2^{30} 7.73 (ring NH^+), pK_3^{30} 10.18 (Et_2N).** Cryst from H_2O (sol 2.8% at room temp) as yellow crystals. Slightly sol in MeOH and EtOH. Antimalarial, antiprotozoal and intercalates DNA. [Wolfe *Antibiot* **3** (Springer-Verlag) 203 *1975*.]

Quisqualic acid (3-[3,5-dioxo-1,2,4-oxadiazolin-2-yl]-L-alanine) *[52809-07-1]* **M 189.1, m 190-191°, $[\alpha]_D^{20}$+17° (c 2, 6M HCl), $pK_{Est(1)}$~ 2.1 (CO_2H), $pK_{Est(2)}$~ 8.9 (NH_2).** It has been purified by ion-exchange chromatography on Dowex 50W (x 8, H^+ form), the desired fractions are lyophilised and recrystd from H_2O-EtOH. It has IR (KBr) ν: 3400-2750br, 1830s, 1775s, 1745s and 1605s cm^{-1}; and ^1H NMR (NaOD/D_2O, pH 13) δ: 3.55-3.57 (1H m, X of ABX, H-2), 3.72-3.85 (2H, AB of ABX, H-3), ^{13}C NMR (D_2O) δ: 50.1t, 53.4d, 154.8s, 159.7s and 171.3s. [Baldwin et al. *J Chem Soc, Chem Commun* 256 *1985*.] It is a quasiqualate receptor agonist [Joels et al. *Proc Natl Acad Sci USA* **86** 3404 *1989*].

Renal dipeptidase **(from porcine kidney cortex)** *[9031-96-3]* **M_r 47,000 [EC 3.4.13.11].** Purified by homogenising the tissue, extracting with Triton X-100, elimination of insoluble material, and ion-exchange, size exclusion and affinity chromatography. [Hitchcock et al. *Anal Biochem* **163** 219 *1987*.]

Restriction enzymes (endonucleases). These are enzymes which cleave double stranded DNA (linear or circular) at specific nucleotide sequences within the DNA strands which are then used for cloning (by ligating bits of DNA sequences together) or used for identifying particular DNA materials, e.g. plasmids, genes etc. A very large number of restriction enzymes are now available commercially and are extensively used in molecular biology. They are highly specific for particular nucleotide arrangements and are sensitive to the reaction conditions, e.g. composition of the medium, pH, salt concentration, temperature etc, which have to be strictly adhered to. The enzymes do not require further purification and the reaction conditions are also provided by the suppliers from which the necessary reaction media can also be purchased (see commercial catalogues).

Retinal (Vitamin A aldehyde), Retinoic acid (Vitamin A acid), Retinyl acetate, Retinyl palmitate see entries in Chapter 4.

Reverse transcriptase (from avian or murine RNA tumour viruses) *[9068-38-6]* **[EC 2.7.7.49].** Purified by solubilising the virus with non-ionic detergent. Lysed virions were adsorbed on DEAE-cellulose or DEAE-Sephadex columns and the enzyme eluted with a salt gradient, then chromatographed on a phosphocellulose column and enzyme activity eluted in a salt gradient. Purified from other viral proteins by affinity chromatography on a pyran-Sepharose column. [Verna *Biochim Biophys Acta* **473** 1 *1977*; Smith *Methods Enzymol* **65** 560 *1980*; see commercial catalogues for other transcriptases.]

Riboflavin *[83-88-5]* **M 376.4, m 295-300°(dec), $[\alpha]_D$ -9.8° (H_2O), -125° (c 5, 0.05N NaOH), pK_1 1.7, pK_2 9.69 (10.2, acidic NH).** Crystd from 2M acetic acid, then extracted with $CHCl_3$ to remove lumichrome impurity. [Smith and Metzler *J Am Chem Soc* **85** 3285 *1963*.] Has also been crystd from water. (See also p. 575.)

Riboflavin-5'-phosphate (Na salt, $2H_2O$) *[130-40-5]* **M 514.4.** See flavin mononucleotide (FMN) on p. 535.

D-(+)-Ribonic acid-γ-lactone *[5336-08-3]* **M 148.12, m 80°, 84-86°, $[\alpha]_D^{20}$ +18.3° (c 5, H_2O).** Purified by recrystn from EtOAc. The *tribenzoate* has **m** 54-56° (from AcOH), $[\alpha]_D^{25}$ +27° (c 2.37, Me_2NCHO) and the *3,5-O-benzylidene* derivative has **m** 230-231.5° (needles from Me_2CO-pet ether), $[\alpha]_D^{25}$ -177° ($CHCl_3$). [Chen and Joulié *J Org Chem* **49** 2168 *1984*; Zinner and Voigt *J Carbohydr Res* **7** 38 *1968*.]

Ribonuclease (from human plasma) *[9001-99-4]* **M_r ~13,700, [EC 3.1.27.5], amorphous.** Purified by $(NH_4)_2SO_4$ fractionation, followed by PC cellulose chromatography and affinity chromatography (using Sepharose 4B to which $(G)_n$ was covalently bonded). [Schmukler et al. *J Biol Chem* **250** 2206 *1975*.]

RNA (ribonucleic acids). Ribonucleic acids are like DNA except that the 2'-deoxy-D-ribose moiety is replaced by a D-ribose moiety and the fourth nucleotide thymidylic acid (T) is replaced by uridylic acid (U). RNA does not generally form complete douplex molecules like DNA, i.e. it is generally monomeric, except in certain viruses. The two main classes of RNA are **messenger-RNA** (mRNA) and **transfer-RNA** (tRNA). mRNA transcribed from the DNA gene followed by the splicing out of the non-coding nucleotides (of the introns) and codes for a specific gene. There are many different tRNAs, at least one of which links to a specific α-amino acid, that bind to the mRNA *via* the ribosome (a set of proteins) to the RNA triplets (three nucleotides) which code for the particular α-amino acids. An enzyme then joins the α-amino acids of two adjacent tRNA-α-amino acid ribosome complexes bound to the mRNA to form a peptide bond. Thus peptide bonds and consequently polypeptides and proteins coded by the DNA *via* the respective mRNA are produced.
Martin et al. [*Biochem J* **89** 327 *1963*] dissolved RNA (5g) in 90mL of 0.1mM EDTA, then homogenised with 90mL of 90% (w/v) phenol in water using a Teflon pestle. The suspension was stirred vigorously for 1h at room temperature, then centrifuged for 1h at 0° at 25000rpm. The lower (phenol) layer was extracted four times with 0.1mM EDTA and the aqueous layers were combined, then made 2% (w/v) with respect to AcOK and 70% (v/v) with respect to EtOH. After standing overnight at -20°, the ppte was centrifuged down, dissolved in 50mL of 0.1mM EDTA, made 0.3M in NaCl and left 3 days at 0°. The purified RNA was then centrifuged down at 10000xg for 30min, dissolved in 100mL of 0.1mM EDTA, dialysed at 4° against water, and freeze-dried. It was stored at -20° in a desiccator. Michelson [*J Chem Soc* 1371 *1959*] dissolved 10g of RNA in water, added 2M ammonia to adjust the pH to 7, then dialysed in Visking tubing against five volumes of water for 24h. The process was repeated three times, then the material after dialysis was treated with 2M HCl and EtOH to ppte the RNA which was collected, washed with EtOH, ether and dried [see commercial catalogues for further examples].

Ricin (toxin from Castor bean *Ricinus communis*) *[A chain 96638-28-7; B chain 96638-29-8]* **M_r ~60,000, amorphous.** Crude ricin, obtained by aqueous extraction and $(NH_4)_2SO_4$ pptn, was chromatographed on a galactosyl-Sepharose column with sequential elution of pure ricin. The second peak was due to ricin agglutinin. [Simmons and Russell *Anal Biochem* **146** 206 *1985*.] Inhibitor of protein synthesis. **EXTREMELY DANGEROUS, USE EXTREME CARE [instructions accompany product].**

Rifampicin (Rifampin) *[13292-46-1]* **M 823.0, m 183-185°, pK_1 1.7, pK_2 7.9.** This macrolide antibiotic crystallises form Me_2CO in red-orange plates. It has UV λ_{max} 237, 255,334, and 475nm (ε 33,200, 32,100, 27,000 and 15,400) at pH 7.38. It is stable in Me_2SO and H_2O. Freely soluble in most organic solvents and slightly soluble in H_2O at pH <6. [Binda et al. *Arzneim.-Forsch* **21** 1907 *1971*.] It inhibits cellular RNA synthesis without affecting DNA [Calvori et al. *Nature* **207** 417 *1965*].

Rifamycin B *[13929-35-6]* **M 755.8, m 300° (darkening at 160-164°), $[\alpha]_D^{20}$ -11° (MeOH), pK_1 2.60, pK_2 7.76.** It forms yellow needles from $^*C_6H_6$. It has solubility in H_2O (0,027%), MeOH (2.62%) and EtOH (0.44%). It has UV λ_{max} 223, 304 and 245nm ($A_{1cm}^{1\%}$ 555, 275 and 220). [Oppolzer and Prelog *Helv Chim Acta* **56** 2287 *1973*; Oppolzer et al. *Experientia* **20** 336 *1964*; X-ray: Brufani et al. *Experientia* **20** 339 *1964*.]

Rifamycin SV sodium salt *[15105-92-7]* **M 719.8, m 300°(darkening >140°), $[\alpha]_D^{20}$ -4° (MeOH), pK_{Est} ~ 7.8.** Yellow orange crystals from Et_2O-pet ether or aq EtOH, very soluble in MeOH, EtOH, Me_2CO and EtOAc, soluble in Et_2O and HCO_3^-, slightly soluble in H_2O and pet ether. Its UV has λ_{max} at 223, 314 and 445nm ($A_{1cm}^{1\%}$ 586, 322 and 204) in phosphate buffer pH 7. [NMR: Bergamini and Fowst *Arzneim.-Forsch* **15** 951 *1965*.]

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