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Saccharides. Resolved by anion-exchange chromatography. [Walberg and Kando *Anal Biochem* **37** 320 *1970*.]

Sarcosine anhydride *[5076-82-4]* **M 142.2, m 146-147°, $pK_{Est(1)}$~-4.2, $pK_{Est(2)}$~-1.9.** Crystd from water, EtOH or ethyl acetate. Dried in vacuum at room temperature.

(-)-Scopolamine hydrobromide $3H_2O$ (6β,7β-epoxy-3α-tropanyl *S*(-)-tropate HBr, hyoscine HBr) *[114-49-8]* **M 438.3, m 193-194°, 195°, 195-199°, $[\alpha]_D^{25}$ -25°(c 5, H_2O), pK^{20} 8.15.** Recrystd from Me_2CO, H_2O or EtOH-Et_2O and dried. Soluble in H_2O (60%) and EtOH (5%) but insol in Et_2O and slightly in $CHCl_3$. The *hydrochloride* has **m** 300° (from Me_2CO). The *free base* is a viscous liquid which forms a crystalline *hydrate* with **m** 59° and $[\alpha]_D^{20}$ -28° (c 2.7, H_2O). Readily hydrolysed in dilute acid or base. [Meinwald *J Chem Soc* 712 *1953*; Fodor *Tetrahedron* **1** 86 *1957*.]

Seleno-DL-methionine (±2-amino-4-methylselanylbutyric acid) *[2578-28-1]* **M 196.1, m 265°(dec), 267-269°(dec), 270° (see pKs of methionine).** Crystallises in hexagonal plates from MeOH and H_2O. [Klosterman and Painter *J Am Chem Soc* **69** 2009 *1949*.] The L-isomer is purified by dissolving in H_2O, adjusting the pH to 5.5 with aqueous NH_3, evaporating to near-dryness, and the residue is washed several times with absolute EtOH till solid is formed and then recrystd from Me_2CO. It has **m** 266-268°(dec), 275°(dec), $[\alpha]_D^{25}$ +18.1°(c 1, N HCl). [Pande et al. *J Org Chem* **35** 1440 *1970*.]

Serotonin hydrochloride (5-HT, 3-[2-aminoethyl]-5-hydroxyindole HCl) *[153-98-0]* **M 212.7, m 167-168°, 178-180°, pK_1^{25} 4.9, pK_2^{25} 9.8 (10.0, NH_2), pK_3^{25} 11.1 (5-OH), pK_4^{25} 18.25 (acidic indole NH).** Purified by recrystn from EtOH-Et_2O or Et_2O to give the *hygroscopic* salt. Store in the dark as it is light sensitive. The *free base* has **m** 84-86° (from Et_2O). The *5-benzyloxy* derivative has **m** 84-86° (from Et_2O). [Ek and Witkop *J Am Chem Soc* **76** 5579 *1954*; HamLin and Fischer *J Am Chem Soc* **73** 5007 *1951*.] The *picrate* $1H_2O$ has **m** 196-197.5° (dec with sintering at 160-165°) after recrystn from Et_2O. Serotonin is a natural neurotransmitter [Chuang *Life Sci* **41** 1051 *1987*].

Sinigrin monohydrate (Myronate K) *[64550-88-5]* **M 415.5, m 125-127°, 127-129°, $[\alpha]_D^{20}$ -17° (c 0.2, H_2O), pK_{Est} <0.** Purified by recryst three times from EtOH and once from MeOH. The *tetraacetate* has **m** 193-195°, $[\alpha]_D^{20}$ -16° (c 0.14, H_2O). [Benn et al. *J Chem Soc, Chem Commun* 445 *1965*; Kjaer et al. *Acta Chem Scand* **10** 432 *1956*; Marsh et al. *Acta Cryst* (*Sect B*) **26** 1030 *1970*.] It is a β-D-thioglucopyranoside substrate for thiogluconidase [MacLeod and Rossiter *Phytochem* **25** 1047 *1986*].

α-Solanine (solan-5-en-3β-yl-[O^3-β-D-glucopyranosyl-O^2-α-L-rhamnopyranosyl-β-D-galacto -pyranoside]) *[20562-02-1]* **M 868.1, m 285°(dec), 286°(dec) (sintering >190°), $[\alpha]_D^{20}$ -58° (c 0.8, pyridine), pK^{15} 6.66.** Recrystd from EtOH, 85% aqueous EtOH, MeOH or aqueous MeOH as *dihydrate* **m** 276-278°. Solubility in H_2O is 25mg/L and 5% in pyridine, but it is very soluble in Et_2O and $CHCl_3$. The *hydrochloride* is gummy or amorphous but has been crystd (**m** ~212° dec). It has insecticidal properties. [Kuhn et al. *Chem Ber* **88** 1492 *1955*.]

Somatostatin *[38916-34-6]* **M 1637.9, $[\alpha]_D^{25}$ -36° (c 0.57, 1% AcOH).** A tetradecapeptide which is purified by gel filtration on Sephadex G-25, eluting with 2N AcOH, and then by liquid partition chromatography on Sepahdex G-25 using *n*-BuOH-AcOH-H_2O (4:1:5) and has R_F = 0.4. It is a brain growth hormone releasing-inhibiting factor which has also been synthesised. [Burgus et al. *Proc Natl Acad Sci USA* **70** 684 *1973*; Sorantakis and McKinley *Biochem Biophys Res Commun* **54** 234 *1973;* Hartridt et al. *Pharmazie* **37** 403 *1982*.]

Spectinomycin dihydrochloride pentahydrate (Actinospectacin) *[21736-83-4]* **M 495.3, m 205-207°(dec), $[\alpha]_D^{20}$ +14.8° (c 0.4, H_2O), pK_1 6.95, pK_2 8.70.** Purified from aqueous Me_2CO and is soluble in H_2O, MeOH and dilute acid and base but only slightly soluble in Me_2CO, EtOH, $CHCl_3$ and $^*C_6H_6$. The *free base* is an amorphous solid, **m** 184-194° with $[\alpha]_D^{20}$ -20° (H_2O). [Wiley et al. *J Am Chem Soc* **93** 2652 *1963*; X-ray: Cochran et al. *J Chem Soc Chem Commun* 494 *1972*.] It is an aminoglycoside antibiotic which interacts with 16S ribosomal RNA [Moazet and Noller *Nature* **327** 389 *1987*]; and is used for the treatment of gonorrhea [Rinehart *J Infect Dis* **119** 345 *1969*].

D-Sphingosine (2*S*,3*S*-D-*erythro*-2-aminooctadec-4*t*-ene-1,3-diol from bovine brain) *[123-78-4]* **M 299.5, m 79-82°, 82° 82.5° (softens at ~70°), $[\alpha]_D^{22}$ -3.4° (c 2, $CHCl_3$), pK_{Est} ~ 8.8.** Purified by recrystn from EtOAc, Et_2O or pet ether (60-80°) It is insoluble in H_2O but is soluble in Me_2CO, EtOH and MeOH. It has IR bands at 1590 and 875 cm^{-1}, and is characterised as the *tribenzoate* **m** 122-123° (from 95% EtOH). [Tipton *Biochem Prep* **9** 127 *1962*.]

Spirilloxanthin *[34255-08-8]* **M 596.9, m 216-218°, λ_{max} 463, 493, 528 nm, $\varepsilon^{1\%}_{1cm}$ 2680 (493 nm) in pet ether (b 40-70°).** Crystd from $CHCl_3$/pet ether, acetone/pet ether, $^*C_6H_6$/pet ether or $^*C_6H_6$. Purified by chromatography on a column of $CaCO_3/Ca(OH)_2$ mixture or deactivated alumina. [Polgar et al. *Arch Biochem Biophys* **5** 243 *1944*.] Stored in the dark in an inert atmosphere, at -20°.

Squalane (Cosbiol, 2,6,10,15,19,23-hexamethyltetracosane, perhydrosqualene) *[111-01-3]* **M 422.8, m -38°, b 176°/0.05mm, 210-215°/1mm, 274°/10mm, ~350°/760mm, d_4^{20} 0.80785, n_D^{20} 1.416.** Purified by fractional distn *in vacuo* or evap distn. Soluble in pet ether, $^*C_6H_6$, Et_2O and $CHCl_3$, slightly sol in alcohols, Me_2CO and AcOH but insol in H_2O [Staudinger and Leupold *Helv Chim Acta* **15** 223 *1932*; Sax and Stross *Anal Chem* **29** 1700 *1951*; Mandai et al. *Tetrahedron Lett* **22** 763 *1981*].

Squalene (*all-trans*-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) *[111-02-4]* **M 410.7, m ~75°, b 203°/0.1mm, 213°/1mm, 285°/25mm, d^{25} 0.8670, n 1.4905.** Crystd repeatedly from Me_2CO (1.4mL/g) using a Dry-ice bath, washing the crystals with cold acetone, then freezing the squalene under vacuum. Squalene was further purified by passage through a column of silica gel or chromatographed on activated alumina, using pet ether as eluent and stored in vac in the dark. Dauben et al. [*J Am Chem Soc* **74** 4321 *1952*] purified squalene *via* its hexachloride and is bactericidal. [Capstack et al. *J Biol Chem* **240** 3258 *1965*; Krishna et al. *Arch Biochem Biophys* **114** 200 *1966*; Heilbron and Thompson *J Chem Soc* 883 *1929*; Karrer et al. *Helv Chim Acta* **13** 1084 *1930*; UV: Farmer et al. *J Chem Soc* 544 *1943*.]

Starch *[9005-84-9]* **M $(162.1)_n$.** Defatted by Soxhlet extraction with Et_2O or 95% EtOH. For fractionation of starch into "amylose" and "amylopectin" fractions, see Lansky et al. [*J Am Chem Soc* **71** 4066 *1949*].

Sterigmatocystin (3a,12c-dihydro-8-hydroxy-6-methoxy-3*H*-furo[3',2',:4,5]furo[2,3-c]-xanthen-7-one) *[10048-13-2]* **M 324.3, m 246°, 247-248°, $[\alpha]_D^{20}$ -398° (c 0.1, $CHCl_3$), pK_{Est} ~ 8.0.** Recrystd from amyl acetate, Me_2CO or EtOH and sublimed *in vacuo*. It has UV λ_{max} at 208, 235, 249 and 329nm (log ε 4.28, 4.39, 4.44 and 4.12). [UV: Bullock et al. *J Chem Soc* 4179, *1962*; UV, IR: Holker and Mulheirn *J Chem Soc Chem Commun* 1576, 1576 *1968*; Birkinshaw and Hammady *Biochem J* **65** 162 *1957*.] This mycotoxin induces bone marrow changes in mice [Curry et al. *Mutation Res* **137** 111 *1984*].

Stigmatellin A (2-[4,6-dimethoxy-3,5,11-trimethyltridecatri-7*t*,9*t*,11*t*-enyl]-8-hydroxy-5,7-dimethoxy-3-methyl-4*H*-1-benzopyran-4-one) *[91682-96-1]* **M 514.6, m 128-130°, $[\alpha]_D^{20}$ +38.5° (c 2.3, MeOH), pK_{Est} ~7 (phenolic OH).** It is stable in aqueous soln at neutral pH but decomposes at pH <5. Purified by recrystn from toluene-hexane). It has UV λ_{max}: nm (ε) 248sh (41000), 258 (59500) 267 (65500), 279 (41400) and 335 (5200) in MeOH; 249sh (45600), 258 (60000), 268 (72700), 277 (54100), 320 (2500) and 370 (3000) in MeOH + 1 drop of N KOH; 243sh (29300), 264 (63200), 274 (64100), 283sh (45800), 329 (4800) and 420 (21000) in MeOH + 6N HCl; and IR ($CHCl_3$) ν: 3550m, 1645chs, 1635ss, 1620ss, 1590s, 1510m and 905m cm^{-1}. It gives colour reactions at 110° with vanillin/H_2SO_4 (grey), Ce(IV)/$(NH_4)_2SO_4$ (yellow) and phosphomolybdate (blue-grey). [Höfle et al. *Justus Liebigs Ann Chem* 1882 *1984*.] It inhibits electron transport [Jagow and Link *Methods Enzymol* **126** 253 *1986*; Robertson et al. *Biochemistry* **32** 1310 *1933*], and has antibiotic properties [Kunze et al. *J Antibiot* **37** 454 *1984*]. The **7*t*,9*t*,11*c*-isomer** is ***Stigmatellin B*.**

Streptomycin sulfate *[3810-74-0]* **M 1457.4, $[\alpha]_D^{20}$ -84.3° (c 3, H_2O), $pK_{Est(1)}$~ 9.5 (MeNH), $pK_{Est(2,3)}$~ 13.4 (guanidino).** Recrystd from H_2O-EtOH, washed with a little EtOH, Et_2O and dried in a vacuum. [UV and IR: Grove and Randall *Antibiotics Monographs* **2** 163 *1855*; Heuser et al. *J Am Chem Soc* **75** 4013 *1953*, Kuehl et al. *J Am Chem Soc* **68** 1460 *1946*; Regna et al. *J Biol Chem* **165** 631 *1946*.] During protein synthesis it inhibits initiation and causes misreading of mRNA [Zierhut et al. *Eur J Biochem* **98** 577 *1979*; Chandra and Gray *Methods Enzymol* **184** 70 *1990*].

Streptonigrin (nigrin, 5-amino-6-[7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolinyl]-4-[2-hydroxy-3,4-dimethoxyphenyl]-3-methyl-2-pyridinecarboxylic acid) *[3930-19-6]* **M 506.5, m 262-263°, 275°(dec), pK 6.3 (1:1 aq dioxane).** Purified by TLC on pH 7-buffered silica gel (made from a slurry of Silica Gel 60 and 400mL of 0.05M phosphate buffer pH 7.0) and eluted with 5% MeOH/$CHCl_3$. The extracted band can then be recrystd from Me_2CO or dioxane as almost black plates or needles. It is soluble in pyridine, Me_2NCHO, aqueous $NaHCO_3$ (some dec), and slightly soluble in MeOH, EtOH, EtOAc and H_2O. It has UV λ_{max} 248, 375-380nm (ε 38400 and 17400). [Weinreb et al. *J Am Chem Soc* **104** 536 *1982*; Rao et al. *J Am Chem Soc* **85** 2532 *1963*.] It is an antineoplastic and causes severe bone marrow depression [Wilson et al. *Antibiot Chemother* **11** 147 *1961*].

Streptozotocin (*N*-[methylnitrosocarbamoyl]-α-D-glucosamine, streptozocin) *[18883-66-4]* **M 265.2, m 111-114°(dec), 114-115°(dec), 115°(dec with evolution of gas), $[\alpha]_D^{20}$ ~+39° (H_2O, may vary due to mutarotation).** Recrystd from 95% EtOH and is soluble in H_2O, MeOH and Me_2CO. It has UV λ_{max} 228nm (ε 6360) in EtOH. The *tetraacetate* has **m** 111-114°(dec), $[\alpha]_D^{25}$ +41° (c 0.78, 95% EtOH) after recrystn from EtOAc. [Herr et al. *J Am Chem Soc* **89** 4808 *1967*; NMR: Wiley et al. *J Org Chem* **44** 9 *1979*.] It is a potent methylating agent for DNA [Bennett and Pegg *Cancer Res* **41** 2786 *1981*].

Subtilisin (from *Bacillus subtilis*) *[9014-01-1]* **[EC 3.4.21.62].** Purified by affinity chromatography using 4-(4-aminophenylazo)phenylarsonic acid complex to activated CH-Sepharose 4B. [Chandraskaren and Dhar *Anal Biochem* **150** 141 *1985*].

Succinyl coenzyme A trisodium salt *[108347-97-3]* **M 933.5.** If it should be purified further then it should be dissolved in H_2O (0.05g/mL) adjusted to pH 1 with 2M H_2SO_4 and extracted several times with Et_2O. Excess Et_2O is removed from the aqueous layer by bubbling N_2 through it and stored frozen at pH 1. When required the pH should be adjusted to 7 with dilute NaOH and used within 2 weeks (samples should be frozen). Succinyl coenzyme A is estimated by the hydroxamic acid method [*J Biol Chem* **242** 3468 *1967*]. It is more stable in acidic than in neutral aqueous solutions. [*Methods Enzymol* **128** 435 *1986*.]

2-Sulfobenzoic cyclic anhydride (2,1-benzoxathiazol-3-one 1,1-dioxide) *[81-08-3]* **M 184.2, m 126-127°, 129.5°, 130°, b 184-186°/18mm.** If the sample has hydrolysed extensively (presence of OH band in the IR) then treat with an equal bulk of $SOCl_2$ reflux for 3h ($CaCl_2$ tube), evaporate and distil residue in a vacuum, then recrystd from $^*C_6H_6$, Et_2O-$^*C_6H_6$ or $CHCl_3$ (EtOH free by passing through Al_2O_3, or standing over $CaCl_2$). [Clarke and Breger *Org Synth* Coll Vol I 495 *1948*.] Used for modifying ζ-amino functions of lysyl residues in proteins [Bagree et al. *FEBS Lett* **120** 275 *1980*]. (see entry on p. 126.)

Syrexin (from bovine liver). Purified by $(NH_4)_2SO_4$ pptn, then by pH step elution from chromatofocusing media in the absence of ampholytes. [Scott et al. *Anal Biochem* **149** 163 *1985*.]

Taurodeoxycholic acid sodium salt monohydrate (*n*-[desoxycholyl)taurine Na salt H_2O) *[1180-95-6]* M 539.7, m 171-175°, $[\alpha]_D^{23}$ +37° (c 1, H_2O), pK 1.4 (free acid).** The salt is recrystd from EtOH-Et_2O. Its solubility in H_2O is 10%. The *free acid* has **m** 141-144°. [Norman *Ark Kemi* **8** 331 *1956*.] It forms mixed micelles and solubilises some membrane proteins [Hajjar et al. *J Biol Chem* **258** 192 *1983*].

Terramycin (oxytetracycline) *[79-57-2]* **M 460.4 (anhy), 496.5 ($2H_2O$), sinters at 182°, melts at 184-185°(dec), $[\alpha]_D^{20}$ -196.6° (equilibrium in 0.1M HCl), -2.1° (equilibrium in 0.1M NaOH).** Crystd (as dihydrate) from water or aqueous EtOH.

2,2:5',2"-Terthiophene *[1081-34-1]* **M 248.4, m 92-93°, 94-95°, 94-94.5°, 94-96°.** Recrystd from MeOH, $^*C_6H_6$, pet ether or MeOH. [UV: Zechmeister and Sease *J Am Chem Soc* **69** 273 *1947*; Steinkopf et al. *Justus Liebigs Ann Chem* **546** 180 *1941*.] Phototoxic nematocide [Cooper and Nitsche *Bioorg Chem* **13** 36 *1985*; *Chan* et al. *Phytochem* **14** 2295 *1975*]. See also Terthiophene on p. 356 in Chapter 4.

Tetracycline *[60-54-8]* **M 444.4, m 172-174°(dec), $[\alpha]_{546}^{20}$ +270° (c 1, MeOH), pK_1^{25} 3.30, pK_2^{25} 7.68, pK_3^{25} 9.69.** Crystd from toluene.

Tetracycline hydrochloride *[64-75-5]* **M 480.9, m 214°(dec), 215-220°, $[\alpha]_D^{25}$ -258° (c 0.5, 0.1N HCl), $[\alpha]_D^{20}$ -245° (c 1, MeOH), pK_1 1.4 (enolic OH), pK_2 7.8 (phenolic OH), pK_3 9.6 (Me_2N).** Recrystd from MeOH + *n*-BuOH or *n*-BuOH + HCl. It is insoluble in Et_2O and pet ether. It has UV λ_{max} at 270 and 366nm in MeOH. [Gottstein et al. *J Am Chem Soc* **81** 1198 *1959*; Conover et al. *J Am Chem Soc* **84** 3222 *1962*.]

6*R*-Tetrahydro-*erythro*-biopterin dihydrochloride (BH_4.2HCl, 6*R*-2-amino-4-hydroxy-6-[{1*R*,2*S*}-1,2-dihydroxypropyl]-5,6,7,8-tetrahydropteridine 2HCl) *[69056-38-8]* **M 316.2, m 245-246°(dec), $[\alpha]_D^{25}$ -6.8° (c 0.67, 0.1N HCl), pK_1 1.37 (pyrimidine$^+$), pK_2 5.6 (5-NH^+), pK_3 10.6 (acidic, 3NH).** Recrystn from HCl enriches BH_4 in the natural 6*R* isomer. Dissolve the salt (~6g) in conc HCl (15mL) under gentle warming then add EtOH (30mL) dropwise, chill and collect the colourless needles (67%, up to 99% if mother liquors are concentrated), and dried *in vacuo* immediately over P_2O_5 and KOH. Stores indefinitely at -20° in a dry atmosphere, Better store in sealed ampoules under dry N_2. It can be recrystd from 6N aqueous HCl. It has UV λ_{max} (2N HCl) 264nm (ε 16770; pH 3.5 phosphate buffer) 265nm (ε 13900); (pH 7.6) 297nm (ε 9500) and 260nm sh (ε 4690). It has been separated from the 6*R*-isomer by HPLC on a Partisil-10SCX column using 30mM ammonium phosphate buffer (pH 3.0) containing 3mM $NaHSO_3$ (2mL/min flow rate; 275nm detector) with retention times of 5.87min (6*R*) and 8.45min (6*S*). It is stable in acidic soln and can be stored for extended periods at -20° in 0.04M HCl. Above pH 7 the neutral species are obtained and these are readily oxidised by oxygen in the solvent to quinonoid species and then further oxidation and degradation occurs at room temperatures. These changes are slower at 0°. The *sulfate salt* can be obtained by recrystn from 2M H_2SO_4 and is less soluble than the hydrochloride salt. The *6R-2,5,1',2'-tetraacetylbiopterin* derivative has **m** 292° (dec) after recrystn from MeOH (100 parts) and $[\alpha]_{589}^{20}$ -144° (c 0.5, $CHCl_3$), $[\alpha]_{589}^{20}$ +12.8° (c 0.39, Me_2SO). [NMR, UV: Matsuura et al. *Heterocycles* **23** 3115 *1985*; Viscontini et al. *Helv Chim Acta* **62** 2577 *1979*; Armarego et al. *Aust J Chem* **37** 355 *1984*.]

Tetrahydrofolic acid dihydrochloride $2H_2O$ (THFA, 6*S*- or 6*RS*- 5,6,7,8-tetrahydrofolic acid 2HCl $2H_2O$, 5,6,7,8-tetrahydropteroyl-L-glutamic acid 2HCl $2H_2O$) *[135-16-0]* **M 544.4, m >200°(dec), $[\alpha]_D^{27}$ +16.9° (H_2O pH 7.0 + 2-mercaptoethanol), pK_1 1.7 (pyrimidine N^+), pK_2 2.4 (10N^+), pK_3 3.5 (α-CO_2H), pK_4 4.9 (γ-CO_2H), pK_5 5.6 (5-NH^+), pK_6 10.4 (acidic, 3NH).** Very high quality material is now available commercially and should be a white powder. It can be dried over P_2O_5 in a vacuum desiccator and stored in weighed aliquots in sealed ampoules. It is stable at room temp in sealed ampoules for many months and for much more extended periods at -10°. When moist it is extremely sensitive to moist air whereby it oxidises to the yellow 7,8-dihydro derivative. In soln it turns yellow in colour as it oxidises and then particularly in the presence of acids it turns dark reddish brown in colour. Hence aqueous solutions should be frozen immediately when not in use. It is always advisable to add 2-mercaptoethanol (if it does not interfere with the procedure) which stabilises it by depleting the soln of O_2. The *sulfate salt* is more stable but then it is much less soluble. The best way to prepare standard solns of this acid is to dissolve it in the desired buffer and estimate the concentration by UV absorption in pH 7 buffer at 297nm (ε 22,000 $M^{-1}cm^{-1}$). If a sample is suspect it is not advisable to purify it because it is likely to deteriorate further as "dry box" conditions are necessary. Either a new sample is purchased or one is freshly prepared from folic acid. It has pKa values of -0.1, 4.3 and 9.0. [Hafeti et al. *Biochem Prep* **7** 89 *1960*; UV: Mathews and Huennekens *J Biol Chem* **235** 3304 *1960*; Osborn and Huennekens *J Biol Chem* **233** 969 *1958*; O'Dell et al. *J Am Chem Soc* **69** 250 *1947*; Blakley *Biochem J* **65** 331 *1957*; Asahi *Yakugaku Zasshi (J Pharm Soc Japan)* **79** 1548 *1959*.]

5,6,7,8-Tetrahydropterin sulfate (2-amino-5,6,7,8-tetrahydropteridin-4-one H_2SO_4) *[20350-44-1]* **M 265, m >200°(dec), pK_1^{25} 1.3 (pyrimidine $^+$), pK_2^{25} 5.6 (5-NH^+), pK_3^{25} 10.6 (acidic, 3NH).** If it has become too strongly violet in colour then it may need reducing again. Best to check the UV absorption in N HCl where it has a peak at ~265nm which drops sharply to zero having no absorption at *ca* 340nm. The presence of absorption at 340nm indicated oxidation to quinonoid or 7,8-dihydropterin. If the absorption is weak then dissolve in the minimum volume of anhydrous trifluoroacetic acid (fume hood) add charcoal, filter, then add one or two drops of N H_2SO_4 followed by dry Et_2O at 0°, allow the white tetrahydro

salt to settle and collect, and wash with dry Et_2O, by centrifugation. Dry the residue in a vacuum desiccator over P_2O_5 and KOH. Store in aliquots in the dark at <0°. It has UV λ_{max} at 265nm (ε 16980) at pH -1.0 (dication); 219nm (ε 23440) and 266nm (ε 12880) at pH 3.5 (monocation); 220nm (ε 18620), [260nm (ε 4270)sh] and 299nm (ε 9330) at pH 8.0 (neutral species); and 218nm (ε 10000), [240nm (ε 5500)sh] and 287nm (ε 5500) at pH 13 (anion). [Blakley *Biochem J* **72** 707 *1959*; Asahi *Yakugaku Zasshi (J Pharm Soc Jpn)* **79** 1557 *1959*; Pfleiderer in *Pterins and Folate* (Benkovic and Blakley Eds) J Wiley Vol 2 p97 *1985*.]

Thiamine monophosphate chloride $1H_2O$ (Aneurine monophosphate chloride) *[532-40-1]* **M 416.8, m 193°(dec), 200°(dec), 200-203°(dec), pK_1 2.40, pK_2 4.80, pK_3 6.27, pK_4 9.65, pK_5 10.20.** Purified by recrystn from aqueous HCl, EtOH slightly acidified with HCl, EtOH-Me_2CO, H_2O, or H_2O-EtOH + Et_2O. Dissolve in a small volume of H_2O and mix with EtOH + Me_2CO (1:1) to give the $HCl.H_2O$ as crystals. Filter, wash with Et_2O and dry in a vacuum. The *chloride hydrochloride* , **m** 215-217°(dec) is obtained when crystd from aqueous HCl. [Wenz et al. *Justus Liebigs Ann Chem* **618** 2280 *1958*, Viscontini et al. *Helv Chim Acta* **34** 1388 *1951*, Leichssenring and Schmidt *Chem Ber* **95** 767 *1962*; McCormick and Wright *Methods Enzymol* **18A** 141, 147 *1970*.]

Thiamphenicol (1*R*,2*R*-2-[2,2-dichloroacetylamino]-1-[4-methanesulfonylphenyl]-propan-1,3-diol) *[15318-45-3 (D-threo), 90-91-5]* **M 356.2, m 163-166°, 165.2-165.6°, 165-166°, $[\alpha]_D^{25}$ +15.6° (c 2, EtOH), pK 7.2.** Recrystd from H_2O or $CHCl_3$. UV λ_{max} 224, 266 and 274nm (ε 13700, 800 and 700) in 95% EtOH. The *1S,2S-isomer [14786-51-7]* has **m** 164.3-166.3° (from H_2O + EtOAc + pet ether) and $[\alpha]_D^{25}$ -12.6° (c 1, EtOH); and the *racemate 1RS,2RS* **Racefenical** *[15318-45-3]* has **m** 181-183° (sinter at 180-183°) from $CHCl_3$-EtOAc-pet ether. [Cutler et al. *J Am Chem Soc* **74** 5475, 5482 *1952*; UV: Nachod and Cutler *J Am Chem Soc* **74** 1291 *1952*; Suter et al. *J Am Chem Soc* **75** 4330 *1953*; Cutler et al. *J Am Pharm Assoc* **43** 687 *1954*.]

Thiazolyl blue tetrazolium bromide (MTT, 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2*H*-tetrazolium bromide) *[298-93-1, 2348-71-2]* **M 414.3, m 171°.** It is recrystd by dissolving in MeOH containing a few drops of HBr and then adding dry Et_2O to complete the crystn, wash the needles with Et_2O and dry in a vacuum desiccator over KOH. [Beyer and Pyl *Chem Ber* **87** 1505 *1954*.]

2-Thiocytosine (4-amino-2-mercaptopyrimidine) *[333-49-3]* **M 127.2, m 236-237°(dec), 285-290°(dec), pK_1^{20} 3.90 (NH_2), pK_2^{20} 11.10 (SH).** It is recrystd from hot H_2O and dried at 100° to constant weight. [Brown *J Appl Chem (London)* **9** 203 *1959*; Russell et al. *J Am Chem Soc* **71** 2279 *1949*.] It is used in transcription and translation studies [Rachwitz and Scheit *Eur J Biochem* **72** 191 *1977*.]

6-Thioguanine *[154-42-7]* **M 167.2, m >300°, pK_1^{23} 8.2 (SH), pK_2^{23} 11.6 (acidic, 9-NH).** Recrystd from H_2O as needles. It has UV λ_{max} at 258 and 347nm (H_2O, pH 1) and 242, 270 and 322nm (H_2O, pH 11). [Elion and Hitchings *J Am Chem Soc* **77** 1676 *1955*; Fox et al. *J Am Chem Soc* **80** 1669 *1958*.] It is an antineoplastic agent [Kataoka et al. *Cancer Res* **44** 519 *1984*].

Thrombin (from bovine blood plasma) *[9002-04-4]* **M_r 32,600 [EC 3.4.4.13].** Purified by chromatography on a DEAE-cellulose column, while eluting with 0.1M NaCl, pH 7.0, followed by chromatography on Sephadex G-200. Final preparation was free from plasminogen and plasmin. [Yin and Wessler *J Biol Chem* **243** 112 *1968*.]
Thrombin from bovine blood was purified by chromatography using *p*-chlorobenzylamino-ε-aminocaproyl agarose, and gel filtration through Sephadex G-25. [Thompson and Davie *Biochim Biophys Acta* **250** 210 *1971*.]
Thrombin from various species was purified by precipitaion of impurities with rivanol. [Miller *Nature* **184** 450 *1959*.]

L-Thyroxine sodium salt ($5H_2O$) *[6106-07-6]* **M 888.9, $[\alpha]_{546}^{20}$ +20° (c 2, 1M HCl + EtOH, 1:4).** Crystd from absolute EtOH and dried for 8h at 30°/1mm.

D-Thyroxine {*O*-[3,5-diiodo-4-oxyphenyl]-3,5-diiodo-D-(-)-tyrosine, 3,3',5,5'-tetra-iodo-D-thyronine} *[51-49-0]* **M 776.9, m 235°(dec), 235-236°(dec), 340°(dec), $[\alpha]_D^{20}$ +4.5° (c 3, aq**

0.2N NaOH in 70% EtOH), $[\alpha]_D^{20}$ -17° (c 2, aq N HCl + EtOH 1:4), pK_1^{25} 2.2 (CO_2H), pK_2^{25} 8.40 (OH), pK_3^{25} 10,1 (NH_2). Recrystd from H_2O as needles or from an ammonical soln by dilution with H_2O, MeOH or Me_2CO. Also purified by dissolving ~6.5 g in a mixture of MeOH (200mL) and 2N HCl (20mL), add charcoal, filter then add NaOAc soln to pH 6 and on standing the thyroxine separates, is washed with MeOH then Me_2CO and dried *in vacuo*. The *N-formyl-D-thyroxine* derivative has **m** 210° and $[\alpha]_{546}^{21}$ -26.9° (c 5, EtOH). The racemate *±-thyroxine* has **m** 256° and is purified in the same way. [Nahm and Siedel *Chem Ber* **96** 1 *1963*; Salter *Biochem J* **24** 471 *1930*.]

L-Thyroxine (*O*-[3,5-diiodo-4-oxyphenyl]-3,5-diiodo-L-(+)-tyrosine, 3,3',5,5'-tetraiodo-D-thyronine) *[51-48-9]* **M 776.9, m 229-230°(dec), ~235°(dec), 237°(dec), $[\alpha]_D^{22}$ -5.1° (c 2, aq N NaOH + EtOH 1:2), $[\alpha]_D^{22}$ +15° (c 5, aq N HCl in 95% EtOH 1:2), $[\alpha]_D^{22}$ +26° (EtOH/1M aq HCl; 1:1) (pK 6.6).** Purification is the same as for the D-isomer above. Likely impurities are tyrosine, iodotyrosine, iodothyroxines and iodide. Dissolve in dilute ammonia at room temperature, then crystd by adding dilute acetic acid to pH 6. The *N-formyl-L-thyroxine* has **m** 214°(dec) and $[\alpha]_{546}^{21}$ +27.8° (c 5, EtOH). [Harington et al. *Biochem J* **39** 164 *1945*; Nahm and Siedel *Chem Ber* **96** 1 *1963*; Reineke and Turner *J Biol Chem* **161** 613 *1945*; Chalmers et al. *J Chem Soc* 3424 *1949*.]

Tissue inhibitor of metalloproteins (TIMP, from human blood plasma), M_r ~30,000. Purified by a [anti-human amniotic fluid-TIMP]-Sepharose immuno-affinity column eluted with 50mM glycine/HCl pH 3.0 buffer that is 0.5M in NaCl then by gel filtn [Cawston et al. *Biochem J* **238** 677 *1986*].

***dl*-α-Tocopherol** (see **vitamin E**) *[59-02-9]* **M 430.7, $A_{1cm}^{1\%}$ 74.2 at 292 nm in MeOH.** Dissolved in anhydrous MeOH (15mL/g) cooled to -6° for 1h, then chilled in a Dry-ice/acetone bath, crystn being induced by scratching with a glass rod.

γ-Tocopherol (3,4-dihydro-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)-2*H*-benzopyran-6-ol) *[54-28-4]* **M 416.7, m -30°, b 200-210°/0.1mm, d_4^{20} 0.951, n_D^{20} 1.505, $[\alpha]_D^{20}$ -2.4° (EtOH).** Purified by distn at high vacuum and stored in dark ampoules under N_2. UV λ_{max} 298nm ($A_{1cm}^{1\%}$ 92.8). It is insoluble in H_2O but soluble in organic solvents. The *allophanate* (used for separating isomers) has **m** 136-138°, $[\alpha]_D^{18}$ +3.4° ($CHCl_3$). [Baxter et al. *J Am Chem Soc* **65** 918*1943*; Emerson et al. *Science* **83** 421 *1936*, *J Biol Chem* **113** 319 *1936*.]

Tolylene-2,4-diisocyanate (toluene-2,4-diisocyanate). *[584-84-9]* **M 174.2, m 19.5-21.5°, 20-22°, 28°, b 126°/11mm, 124-126°/18mm, 250°/760mm.** It is purified by fractionation in a vacuum and should be stored in a dry atmosphere. It is soluble in organic solvents but reacts with H_2O, alcohols (slowly) and amines all of which could cause explosive polymerisation. It darkens on exposure to light. It has a sharp pungent odour, is **TOXIC** and is **IRRITATING TO THE EYES.** [Siefken *Justus Liebigs Ann Chem* **562** 75, 96, 127 *1949*; Bayer *Angew Chem* **59** 257 *1947*.] It is a reagent for covalent crosslinking of proteins [Wold *Methods Enzymol* **25** 623 *1972*.]

Tomatidine (5α,20β,22α,25β,27-azaspirostan-3β-ol) *[77-59-8]* **M 415.7, m 202-206°, $[\alpha]_D^{20}$ +5.9° (c 1, MeOH), $[\alpha]_D^{20}$ +8° ($CHCl_3$).** Forms plates from EtOAc. Also purified by dissolving 80mg in $^*C_6H_6$ and applying to an Al_2O_3 column (3.0g) and eluting with $^*C_6H_6$, evaporating and recrystallising three times from EtOAc. The *hydrochloride* has **m** 265-270° from EtOH and $[\alpha]_D^{25}$ -5° (MeOH). [IR: Uhle *J Am Chem Soc* **83** 1460 *1961*; Kessar et al. *Tetrahedron* **27** 2869 *1971* ; Schreiber and Adams *Experientia* **17** 13 *1961*.]

Tomatine (22*S*,25*S*-3β,β-lycotetraosyloxy-5α-spirosolan) *[17406-45-0]* **M 1034.2, m 263-268°(dec), 290-291°(evac capillary), 283.5-287°(dec), 272-277°(dec), 300-305°(dec), $[\alpha]_D^{20}$ -18° to -34° (c 0.55, pyridine).** Recrystd from MeOH, EtOH, aqueous EtOH or dioxane + NH_3. It is almost insoluble in pet ether, Et_2O or H_2O. [Reichstein *Angew Chem* **74** 887 *1962*.]

***N*-Tosyl-L-lysine chloromethyl ketone (3*S*-1-chloro-3-tosylamino-7-amino-2-heptanone HCl)** *[4272-74-6]* **M 369.3, m 150-153°(dec), 156-158°(dec), ~165°(dec), $[\alpha]_D^{20}$ -7.3° (c 2, H_2O), pK_{Est} ~ 10.6 (7-NH_2).** The hydrochloride slowly crystallises from a conc soln in absolute EtOH,

thinned with EtOH-Et_2O for collection and dried *in vacuo*. It is a suicide enzyme inhibitor [Matsuda et al. *Chem Pharm Bull Jpn* **30** 2512 *1982*; Shaw et al. *Biochemistry* **4** 2219 *1965*].

Transferrin (from human or bovine serum) *[11096-37-0]* **M_r~80,000.** Purified by affinity chromatography on phenyl-boronate agarose followed by DEAE-Sephacel chromatography. The product is free from haemopexin. [Cook et al. *Anal Biochem* **149** 349 *1985*; Aisen and Listowsky *Ann Rev Biochem* **49** 357 *1980*.]

Trehalase (from kidney cortex) *[9025-52-9]* **[EC 3.2.1.28].** Purified by solubilising in Triton X-100 and sodium deoxycholate, and submitting to gel filtration, ion-exchange chromatography, conA-Sepharose chromatography, phenyl-Sepharose CL-4B hydrophobic interaction chromatography, Tris-Sepharose 6B affinity and hydrolyapatite chromatography. Activity was increased 3000-fold. [Yoneyama *Arch Biochem Biophys* **255** 168 *1987*.]

Trifluoperazine dihydrochloride (10-[3-{4-methyl-1-piperazinyl}propyl]-2-trifluoro-methyl-phenothiazine 2HCl) *[440-17-5]* **M 480.4, m 240-243°, 242-243°, pK_1 3.9, pK_2 8.1.** Recrystd from abs EtOH dried *in vacuo* and stored in tightly stoppered bottles because it is *hygroscopic*. It is soluble in H_2O but insoluble in $^*C_6H_6$, Et_2O and alkaline aqueous soln. It has UV λ_{max} at 258 and 307.5nm (log ε 4.50 and 3.50) in EtOH (neutral species). [Craig et al. *J Org Chem* **22** 709 *1957*.] It is a calmodulin inhibitor [Levene and Weiss *J Parmacol Exptl Ther* **208** 454 *1978*], and is a psychotropic agent [Fowler *Arzneim.-Forsch* **27** 866 *1977*].

T4-RNA ligase (from bacteriophage-infected *E.coli*) M_r 43,500 [EC 6.5.1.3 for RNA lyase**].** Purified by differential centrifugation and separation on a Sephadex A-25 column, then through hydroxylapatite and DEAE-glycerol using Aff-Gel Blue to remove DNAase activity. (Greater than 90% of the protein in the enzyme preparation migrated as a single band on gradient polyacrylamide gels containing SDS during electrophoresis.) [McCoy et al. *Biochim Biophys Acta* **562** 149 *1979*.]

Tubercidin (7-deazaadenosine) *[69-33-0]* **M 266.3, m 247-248°, $[\alpha]_D^{17}$ -67° (50% aq AcOH), pK^{10} 5.2-5.3.** Forms needles from hot H_2O. It is soluble in H_2O (0.33%), MeOH (0.5%) and EtOH 0.05%). It has UV λ_{max} 270nm (ε 12100) in 0.001N NaOH. The *picrate* has **m** 229-231°(dec). [Tolman et al. *J Am Chem Soc* **91** 2102 *1969*; Mizuno et al. *J Org Chem* **28** 3329 *1963*, IR: Anzai et al. *J Antibiot (Japan)* **[9] 10** 201 *1957*.]

Tunicamycin *[11089-65-9]* **m 234-235°(dec), $[\alpha]_D^{20}$ +52° (c 0.5, pyridine), pK_{Est} ~ 9.4.** The components are purified by recrystallising 3 times from hot glass-distilled MeOH and the white crystals are dissolved in 25% aqueous MeOH and separated on a Partisil ODS-10μ column (9.4 x 25 cm) [Magnum-9 Whatman] using a 260 nm detector. The column was eluted with MeOH:H_2O mixture adjusted to 1:4 (v/v) then to 2:4 (v/v). The individual components are recovered and lyophilised. Ten components were isolated and all were active (to varying extents) depending on the lengths of the aliphatic side-chains. The mixture has UV λ_{max} 205 and 260nm ($A_{1cm}^{1\%}$ 230 and 110). Stable in H_2O at neutral pH but unstable in acidic soln. It inhibits protein glycosylation. [Mahoney and Duskin *J Biol Chem* **254** 6572 *1979*; Elnein *Trends Biochem Sci* **6** 219 *1981*; Takatsuki *J Antibiot* **24** 215 *1971*.]

Ubiquinol-cytochrome c reductase (from beef heart mitochondria)

[9027-03-6] **[EC 1.10.2.2]**. Purified in Triton X-100 by solubilising the crude enzyme with Triton X-100, followed by hydroxylapatite and gel chromatography. The minimum unit contains nine polypeptide subunits of M_r 6000 - 49000 kD. [Engel et al. *Biochim Biophys Acta* **592** 211 *1980*.]

Uracil, uridine and uridine nucleotides. Resolved by ion-exchange chromatography AG1 (Cl^- form). [Lindsay et al. *Anal Biochem* **24** 506 *1968*.]

Uridine 5'-diphosphoglucose pyrophosphorylase (from rabbit skeletal muscle) *[9029-22-6]* **M_r 350,000, [EC 2.7.7.9].** Purified by two hydrophobic chromatographic steps and gel filtration. [Bergamini et al. *Anal Biochem* **143** 35 *1984*.] Also purified from calf liver by $(NH_4)_2SO_4$ (40-58%) pptn, $Ca_3(PO_4)_2$ gel filtration, DEAE-cellulose chromatography and recrystn by dialysis against increasing concentrations of $(NH_4)_2SO_4$ (from 10%) in 0.02M TEA (at 2.5% increments) until at 20% $(NH_4)_2SO_4$ it crystallises out [Hansen et al. *Methods Enzymol* **8** 248 *1966*].

Uridine 5'-(1-thio) monophosphate *[15548-52-4, 18875-72-4 (Abs Stereochem specified)]* **and Uridine 5'-(α-thio) diphosphate** *[RS(α-P) 27988-67-6; R(α-P) 72120-52-6]* **, $pK_{Est(1)}$~ 6.4, $pK_{Est(2)}$~ 9.5** The Et_3N salt was purified by dissolving ~4g in 500mL of H_2O (add a drop or two of Et_3N if it does not dissolve) and chromatographed by applying to a column (3 x 30cm) of DEAE-Sephadex A-25 and eluted with a 1.4L linear gradient of $Et_3NH.HCO_3$ from 0.05 to 0.55M, pH 7.8 and 4°. The product eluted between 0.2-0.3M $Et_3N.HCO_3$. Pooled fractions were evaporated and the residue was twice taken up in EtOH and evaporated to dryness to remove the last traces of $Et_3NH.HCO_3$. ^{31}P NMR: P_α is a doublet at -40.81 and -40.33, and P_β at 7.02ppm, $J_{\alpha,\beta}$ 32.96Hz. [*Biochemistry* **18** 5548 *1979*.]

Uridylic acid (di-Na salt) *[27821-45-0]* **M 368.2, m 198.5°, pK_3^{25} 6.63, pK_4^{20} 9.71.** Crystd from MeOH.

Urokinase (from human urine) *[9039-53-6]* **M_r 53,000, [EC 3.4.21.31].** Crystn of this enzyme is induced at pH 5.0 to 5.3 (4°) by careful addition of NaCl with gentle stirring until the soln becomes turbid (silky sheen). The NaCl concentration is increased gradually (over several days) until 98% of saturation is achieved whereby the urokinase crystallises as colourless thin brittle plates. It can be similarly recrystd to maximum specific activity [104K CTA units/mg of protein (Sherry et al. *J Lab Clin Med* **64** 145 *1964*)]. [Lesuk et al. *Science* **147** 880 *1965*; NMR: Bogusky et al. *Biochemistry* **28** 6728 *1989*.] It is a plasminogen activator [Gold et al. *Biochem J* **262** *1989*].

(+)-Usnic acid (2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyldibenzofuran-1,3(2*H*,9b*H*)-dione) *[7562-61-0, 125-46-2]* **M 344.3, m 201-204°, 203-206°, $[\alpha]_{546}^{20}$ +630° (c 0.7, $CHCl_3$), pK_1 4.4, pK_2 8.8, pK_3 10.7.** This very weak acid is the natural form which is recrystd from Me_2CO, MeOH or $*C_6H_6$. At 25° it is soluble in H_2O (<0.01%), Me_2CO (0.77%), EtOAc (0.88%), $MeOCH_2CH_2OH$ (0.22%) and furfural (7.32%). [Curd and Robertson *J Chem Soc* 894 *1937*; Barton and Brunn *J Chem Soc* 603 *1953*; resolution: Dean et al. *J Chem Soc* 1250 *1953*; synthesis: Barton et al. *J Chem Soc* 538 *1956*.]

(-)-Usnic acid (2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyldibenzofuran-1,3(2*H*,9b*H*)-dione) *[6159-66-6, 7562-61-0]* **M 344.3, m 201-204°, 204°, $[\alpha]_D^{20}$-495° (c 0.9, $CHCl_3$).** Properties almost similar to those of the preceding entry.

Ustilagic acid (Ustizeain B, di-D-glucosyldihydroxyhexadecanoic acid) *[8002-36-6]* **M ~780, m 146-147°, $[\alpha]_D^{23}$ +7° (c 1, pyridine), pK ~ 4.9.** It is a mixture of partly acetylated di-D-glucosyldihydroxyhexadecanoic acid which crysts from diethyl ether. Also purified from the culture by dissolving in hot MeOH, filtering and concentrating by blowing a current of air until the soln becomes turbid, then heating to 50° and adding 4 vols of H_2O (also at 50°) and allowing to cool very slowly. Filter off the white solid and dry in air. [Lemieux et al. *Can J Chem* **29** 409, 415 *1951*; *Can J Biochem Physiol* **33** 289 *1955*.]

Valinomycin

(Potassium ionophore I) *[2001-95-8]* **M 111.3, m 186-187°, 190°, $[\alpha]_D^{20}$ +31.0° (c 1.6, $*C_6H_6$).** Recryst from dibutyl ether or Et_2O. Dimorphic, modification A crystallises from *n*-octane, and modification B crystallises from $EtOH/H_2O$. Soluble in pet ether, $CHCl_3$, AcOH, BuOAc and Me_2CO. [*J Am Chem Soc* **97** 7242 *1975*; UV, IR and NMR see *Chem Ber* **88** 57 *1955*.]

(±)-Verapramil hydrochloride (5-[*N*-{3,4-dimethoxyphenylethyl}methylamino]-2-[3,4-dimethoxyphenyl]-2-isopropylvaleronitrile HCl) *[23313-68-0]* **M 491.1, m 138.5-140.5°,**

pK_{Est} ~ 10.6. Purified by dissolving in EtOH, filtering (if insoluble particles are present) and adding Et_2O, filtering the salt, washing with Et_2O and drying *in vacuo*. It has the following solubilities: hexane (0.001%), CH_2Cl_2 (~10%), MeOH (~10%) EtOH (20%) and H_2O (8.3%). It has UV λ_{max} 232 and 278nm. The *free base* is a viscous yellow oil **b** 243-246°/0.01mm (n_D^{25} 1,5448) and is almost insol in H_2O but sol in organic solvents. It is a Ca channel antagonist and is a coronary vasodilator. [Ramuz *Helv Chim Acta* **58** 2050 *1975*; Harvey et al. *Biochem J* **257** 95 *1989*.]

Veratridine (3-veratroylveracevine) *[71-62-5]* **M 673.8, pK_{Est} ~7 (quinolizidine N).** An alkaloid neurotoxin purified from veratrine. [McKinney et al. *Anal Biochem* **153** 33 *1986*.]

Vinblastine sulfate (vincaleucoblastine) *[143-67-9]* **M 909.1, m 284-285°, $[\alpha]_D^{25}$ -28° (c 1, MeOH), pK_1 5.4, pK_2 7.4.** Purified by recrystn from H_2O and dried *in vacuo*. [Neuss et al. *J Am Chem Soc* **86** 1440 *1964*.] The *free base* is recrystd from MeOH or EtOH and has **m** 210-212°, 211-216°, $[\alpha]_D^{25}$ +42° ($CHCl_3$); and has UV λ_{max} 214 and 259nm (log ε 4.73 and 4.21). The *dihydrochloride dihydrate* has **m** 244-246°. [Bommer et al. *J Am Chem Soc* **86** 1439 *1964*.] It is a monoamine oxidase inhibitor [Keun Son et al. *J Med Chem* **33** 1845 *1990*].

Vincristine sulfate (22-oxovincaleucoblastine sulfate) *[2068-78-2]* **M 925.1, m 218-220°, $[\alpha]_D^{25}$ +26.2° (CH_2Cl_2), pK_1 5.0, pK_2 7.4 (in 33% aq Me_2NCHO).** Recryst from MeOH. It has UV λ_{max} 220, 255 and 296nm (log ε 4.65, 4.21 and 4.18). It is a monoamine oxidase inhibitor and is used in cancer research [Son et al. *J Med Chem* **33** 1845 *1990*; Horio et al. *Proc Natl Acad Sci USA* **85** 3580 *1988*].

Viomycin sulfate (Viocin, Tuberactinomycin B) *[37883-00-4]* **M 685.7, m 266°(dec), $[\alpha]_D^{17}$ -29.5° (c 1, H_2O), pK_1 7.2 (8.2), pK_2 10.3.** Crystd from H_2O-EtOH and dried in a vacuum. Dry material is *hygroscopic* and should be stored dry. The UV has λ_{max} at 268 and 285nm (log ε 4.4 and 4.2) in H_2O. [Kitigawa et al. *Chem Pharm Bull Jpn* **20** 2176 *1972*.] The *hydrochloride* forms *hygroscopic* plates with **m** 270°(dec), $[\alpha]_D^{18}$ -16.6° (c 1, H_2O) with λ_{max} 268nm (log ε 4.5) in H_2O; 268nm (log ε 4.4) in 0.1N HCl and 285nm (log ε 4.3) in 0.1N NaOH.

Vitamin A acid [Retinoic acid, 3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)-2,4,6,8-nonatetraen-1-oic acid] *[302-79-4]* **M 300.4, m 180-181°, 180-182°, pK_{Est} ~ 4.2.** Purified by chromatography on silicic acid columns, eluting with a small amount of EtOH in hexane. Dissolve in Et_2O, wash with H_2O, dry (Na_2SO_4), evaporate and the solid residue crystd from MeOH (0.53g /3.5mL MeOH to give 0.14g) or EtOH. Also recrystd from *i*-PrOH, or as the *methyl ester* from MeOH. UV in MeOH has λ_{max} 351nm (ε 45,000). 9-*Cis*-acid forms yellow needles from EtOH, **m** 189-190°, UV in MeOH has λ_{max} 343nm (ε 36,500) and 13-*cis*-acid forms red-orange plates from *i*-PrOH, **m** 174-175°, UV has λmax 345nm (ε 39,800). Store in the dark, in an inert atmosphere, at 0° [Robeson et al. *J Am Chem Soc* **77** 4111 *1955*].

Vitamin A alcohol (retinol) *[68-26-8]* **M 286.5, $A_{1cm}^{1\%}$ (λmax)(*all-trans*) 1832 (325 nm), (*13-cis*) 1686 (328nm), (*11-cis*) 1230 (319 nm), (*9-cis*) 1480 (323 nm), (*9,13-di-cis*) 1379 (324 nm), (*11,13-di-cis*) 908 (311 nm) in EtOH.** Purified by chromatography on columns of water-deactivated alumina eluting with 3-5% acetone in hexane. Separation of isomers is by TLC plates on silica gel G, developed with pet ether (low boiling)/methyl heptanone (11:2). Stored in the dark, under nitrogen, at 0°, as in diethyl ether, acetone or ethyl acetate. [See Gunghaly et al. *Arch Biochem Biophys* **38** 75 *1952*.]

Vitamin A aldehyde [all-*trans*-retinal; 3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)-2,4,6,8-nonatetraen-1-al] *[116-31-4]* **M 284.4, m 61-64°.** Separated from retinol by column chromatography on water-deactivated alumina. Eluted with 1-2% acetone in hexane, or on TLC plates of silica gel G development. It crystallises from pet ether or *n*-hexane as yellow-orange crystals, and the UV in hexane has λ_{max} 373nm ($A_{1cm}^{1\%}$ 1,548) [368nm (ε 48000)]. It is an **irritant** and is light sensitive. Store in sealed ampoules under N_2. The **semicarbazone** forms yellow crystals from $CHCl_3$-Et_2O or EtOH, **m** 199-201°(dec). The 9-*cis-isomer [514-85-2]* and the 13-*cis-isomer [472-86-6]* [λ_{max} 375nm (ε 1250) in EtOH] are also available commercially.

Vitamin B_1 Hydrochloride [Aneurine hydrochloride, Thiamine hydrochloride, 3{(4-amino-2-methyl-5-pyrimidinyl)methyl}-4-methylthiazolium chloride monohydrochloride] *[67-03-8]* **M 337.3, m 248°(dec), 249-250°, monohydrate m 135°(dec), pK_1^{25} 4.8, pK_2^{25} 9.2.** Crystallises from 95% EtOH (sol, *ca* 1%). The monohydrate is dehydrated by drying at 100° *in vacuo* over H_2SO_4, but is *hygroscopic* and picks up one mol. of H_2O readily. It can be sterilised at 100° if the pH of the solution is below 5.5. The *nitrate* has **m** 196-200°(dec) and is more stable than the hydrochloride. The *picrolonate* crystallises from H_2O and is dimorphic, **m** 164-165° and 228-229°(dec). [Todd and Bergel *J Chem Soc* 364, 367 *1937*; *J Am Chem Soc* **58** 1063, 1504 *1936*, **59** 526 *1937*.]

Vitamin B_2 [Riboflavin, Lactoflavin, 6,7-dimethyl-9-(D-1'-ribityl)isoalloxazine] *[83-88-5]* **M 376.4, m 278-282°(dec with darkening at 240°), 281-282°, $[\alpha]_D^{25}$ -112° to -122° (c 2.5, 0.02M NaOH), $[\alpha]_D^{20}$ -59° (c 0.23, AcOH), pK_1 1.7, pK_2 9.69 (10.2).** It crystallises from H_2O as a yellow-orange powder in three different forms with differing amounts of H_2O. It melts if placed in an oil bath at 250°, but decomposes at 280° if heated at a rate of 5°/min. Solubility in H_2O is 1g in 3000-15000mL depending on the crystal structure. Sol in EtOH at 25° is 4.5mg in 100mL. Store in the dark because it is decomposed to lumichrome by UV light.

Vitamin B_6 hydrochloride (adermine, pyridoxine HCl, 3-hydroxy-4,5-bis[hydroxymethyl]-2-methylpyridine HCl) *[58-56-0]* **M 205.6, m 208-208.5°, 208-209°(dec), 209-210°(dec), 205-212° (sublimes), pK_1^{25} 5.0 (3-OH), pK_2^{25} 8.96 (pyridinium$^+$).** Purified by recrystn form EtOH-Me_2CO, *n*-BuOH or MeOH-Et_2O. Its solubility in H_2O is 22% and in EtOH it is 1.1%. It is insoluble in Et_2O and $CHCl_3$. Acidic aqueous solns are stable at 120°/30 min. The *free base* has **m** 159-160° after recrystn from Me_2CO and sublimation at 140-145°/0.0001mm. It has UV λ_{max} at 290nm (ε 84000) in 0.1N aqueous HCl and 253 and 325nm (ε 3700 and 7100). [Khua and Wendt *Chem Ber* **71** 780 *1938, 72* 311 *1939;* Harris and Folkers *J Am Chem Soc* **61** 1242 *1939*; Harris et al. *J Am Chem Soc* **62** 3198 *1940*.] See also **Pyridoxal-5'-phosphate H_2O** above.

Vitamin B_{12} (cyanocobalamine, α-[5,6-dimethylbenzimidazolyl]cyano cobamide) *[68-19-9]* **M 1355.4, m darkens at 210-220° and does not melt below 300°, $[\alpha]_{656}^{23}$ -59° (H_2O).** Crystd from de-ionized H_2O, solubility in H_2O is 1g/80g and dried under vacuum over $Mg(ClO_4)_2$. The dry red crystals are *hygroscopic* and can absorb ~12% of H_2O. A soln at pH 4.5-5 can be autoclaved for 20min at 120° without dec. Aqueous solns are stabilised by addition of $(NH_4)_2SO_4$. [Golding *Comprehensive Organic Chem* Vol 5 (Ed. Haslam; Pergamon Press, NY, 1979) pp 549-584.]
Alternatively an aqueous soln of the coenzyme was concentrated, if necessary in a vacuum at 25° or less, until the concentration was 0.005 to 0.01M (as estimated by the OD at 522 nm of an aliquot diluted with 0.01M K-phosphate buffer pH 7.0). If crystals begin to form on the walls of the container they should be re-dissolved with a little H_2O. The concentrated soln is placed in a glass stoppered flask and diluted with 5vols of Me_2CO. After 2-3h at 3° it is centrifuged (10,000xg/10 min) in Me_2CO-insol plastic tubes to remove some amorphous ppte. The clear supernatant is inoculated with a small crystal of the vitamin and allowed to crystallise overnight at 3°. Crystals are formed on the walls and the bottom of the container. A further 2vols of Me_2CO are added and set aside at 3° to further crystallise. Crystallisation is followed by observing the OD_{522} of the supernatant. When the OD falls to 0.27 then *ca* 94% of the crystals have separated. The supernatant is decanted (saved for obtaining a second crop) and the crystals are washed with a little cold 90% aqueous Me_2CO (2 x), 100% Me_2CO (2 x), Et_2O (2 x) at which time the crystals separated from the glass walls. Allow to settle and remove residual Et_2O with a stream of dry N_2. The process can be repeated if necessary. The crystals can be dried in air or in a vacuum for 2h over silica gel at 100° with an 8-9% weight loss. [Barker et al. *Biochem Prep* **10** 33 *1963*.] This material gives a single spot of paper chromatography [see Weissbach et al. *J Biol Chem* **235** 1462 *1960*.] The vitamin is soluble in H_2O (16.4mM at 24°, 6.4mM at 1°), in EtOH and PhOH but insol in Me_2CO, Et_2O, CH_2Cl_2 and dioxane. UV: λ_{max} 260, 375 and 522nm (ε 34.7 x 10^6, 10.9 x 10^6 and 8.0 x 10^6 / mole) in H_2O. The dry crystals are stable for months in the dark, but aqueous solns decompose on exposure to VIS or UV light or alkaline CN^-, but stable in the dark at pH 6-7. The vitamin is inactivated by strong acids or alkalies. [Barker et al. *J Biol Chem* **235** 480 *1960*; see also *Vitamin B_{12}* (Zagalak and Friedrich Eds) W de Gruyter, Berlin *1979*.]

Vitamin C see **ascorbic acid** entry on p. 116 in Chapter 4.

Vitamin D_2 *[50-14-6]* **M 396.7, m 114-116°, $[\alpha]_{546}^{20}$ +122° (c 4, EtOH)** Converted into their 3,5-dinitrobenzoyl esters, and crystd repeatedly from acetone. The esters were then saponified and the free vitamins were isolated. [Laughland and Phillips *Anal Chem* **28** 817 *1956.*]

Vitamin D_3 *[67-97-0]* **384.6, m 83-85°, $[\alpha]_{546}^{20}$ +126° (c 2, EtOH).** Converted into their 3,5-dinitrobenzoyl esters, and crystd repeatedly from acetone. The esters were then saponified and the free vitamins were isolated. [Laughland and Phillips *Anal Chem* **28** 817 *1956.*]

Vitamin E (2*R*,4'*R*,8'*R*-α-tocopherol, natural active isomer) *[59-02-9]* **M 430.7, m 2.5-3.5°, b 200-220°/0.1mm, 200°/0.005mm, d_4^{25} 0.950, n_D^{25} 1.5045, $[\alpha]_D^{25}$ +3.58° (c 1.1, $^*C_6H_6$).** Viscous yellow oil which is distd at high vacuum. It has λ_{max} 294nm ($E_{1cm}^{1\%}$ 71). It is oxygen and light sensitive and is best stored as its stable acetate which is purified by evaporative distn at **b** 180-200°(bath temp)/0.7mm, $[\alpha]_D^{25}$ +3.3° (c 5.1, EtOH). [NMR: Cohen et al. *Helv Chim Acta* **64** 1158 *1981*; Burton and Ingold *Acc Chem Res* **19** 194 *1986*; Karrer et al. *Helv Chim Acta 21* 520 *1938.*]

Vitamin E acetate (DL-α-tocopheryl acetate) *[7695-91-2]* **M 472.8, m -27.5°, b 194-196°/0.01mm, 222-224°/0.3mm, d_4^{20} 0.958, n_D^{20} 1.4958.** It is a viscous liquid which is purified by distn under high vacuum in an inert atm and stored in sealed ampoules in the dark. It is considerably more stable to light and air than the parent unacetylated vitamin. It is insoluble in H_2O but freely soluble in organic solvents. All eight stereoisomers have been synthesised. The commercially pure *d-α–tocopheryl acetate* (2R,4'R,8'R) has **b** 180-200°/0.7mm and $[\alpha]_D^{20}$ +3.9° (c 5, EtOH). [Cohen et al. *Helv Chim Acta* **64** 1158 *1981.*]

Vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone) *[84-80-0]* **M 450.7, m -20°, b 141-140/0.001mm, b 140-145°/10^{-3} mm, d_{25}^{25} 0.967, n_D^{25} 1.527, $[\alpha]_D^{20}$ -0.4° (c 57.5, $^*C_6H_6$).** Yellow viscous oil, which can be distd at high vacuum practically unchanged. Insoluble in H_2O, but soluble in common organic solvents. Store in the dark under N_2, oxygen sensitive. $A_{1cm}^{1\%}$ 328 at 248nm. [*J Am Chem Soc* **61** 2557 *1939,* **76** 4592 *1954*; *Helv Chim Acta* **27** 225 *1954.*]

Vitamin K_3 (2-methyl-1,4-naphthoquinone, Menadione, Menaphthone) *[58-27-5]* **M 172.2, m 105-106°, 105-107°.** Recrystd from 95% EtOH, or MeOH after filtration. Bright yellow crystals which are decomposed by light. Solubility in EtOH is 1.7% and in $^*C_6H_6$ it is 10%. It **IRRITATES** the mucous membranes and skin. [Fieser *J Biol Chem* **133** 391 *1940.*]

Xanthine

(2,6-dihydroxypurine, purine-2,6(1*H*,3*H*)dione) *[69-89-6]* **M 152.1, pK_1 0.8 [protonation of imidazole 7(9)NH], pK_2 7.44 [monoanion 1(3)NH], pK_3 11.12 [dianion 1,3-N^{2-}].** The monohydrate separates in a microcryst form on slow acidification with acetic acid of a solution of xanthine in dil NaOH. Also ppted by addition of conc NH_3 to its soln in hot 2N HCl (charcoal). After washing with H_2O and EtOH, it is dehydrated on heating above 125°. Sol in H_2O is 1 in 14,000 at 16° and 1 in 1,500 and separates as plates from boiling H_2O. It has no **m**, but the *perchlorate* has **m** 262-264° [Lister *Heterocyclic Compounds, Fused Pyrimidines—Purines Part II,* Ed. Brown, J.Wiley & Sons, *1971*].

Xanthopterin monohydrate (2-amino-4,6-dihydroxypteridine, 2-amino-pteridin-4,6(1*H*,5*H*)-dione) *[5979-01-1 (H_2O), 119-48-8 (anhydr)]* **M 197.2, m <300°, pK_1 1.6 (basic), pK_2 6.59 (acidic), pK_3 9.31 (acidic)(anhydrous species), and pK_1 1.6 (basic), pK_2 8.65 (acidic), pK_3 9.99 (acidic)(7,8-hydrated species).** Purification as for isoxanthopterin. Crystd by acidifying an ammoniacal soln, and collecting by centrifugation followed by washing with EtOH, ether and drying at 100° *in vacuo*. Paper chromatography R_F 0.15 (*n*-PrOH, 1% aq NH_3, 2:1), 0.36 (*n*-BuOH,AcOH, H_2O, 4:1:1) and 0.47 (3% aq NH_3). [Inoue and Perrin *J Chem Soc* 260 *1962*; Inoue *Tetrahedron* **20** 243 *1964*; see also Blakley *Biochemistry of Folic Acid and Related Pteridines* North Holland Publ Co, Amsterdam 1969.]

Xanthotoxin (Methoxalen, 9-methoxyfuro[3,2-g][1]benzopyran-7-one) *[298-81-7]* **M 216.2, m 146-148°, 148°, 148-149°.** Purified by recrystn from $^{*}C_6H_6$-pet ether (b 60-80°) as silky needles, EtOH-Et_2O as rhombic prisms or hot H_2O as needles. It is soluble in aqueous alkali due to ring opening of a lactone but recyclises upon acidification. It has UV λ_{max} in EtOH at 219, 249 and 300nm (log ε 4.32, 4.35 and 4.06) and ^1H NMR in $CDCl_3$ with δ at 7.76 (d, 1H, *J* 10 Hz), 7.71 (d, 1H, *J* 2.5 Hz), 7.38 (s, 1H), 6.84 (d, 1H, *J* 2.5 Hz), 6.39 (d, 1H, *J* 10 Hz) and 4.28 (s, 3H). [Nore and Honkanen *J Heterocycl Chem* **17** 985 *1980*.] It is a DNA intercalator and is used in the treatment of dermal diseases [Tessman et al. *Biochemistry* **24** 1669 *1985*.]

Xylanase (from *Streptomyces lividans*) *[37278-89-0]* **M_r 43,000 [EC 3.2.1.8].** Purified by anion-exchange chromatography on an Accell QMA column and finally by HPLC using a ProteinPak DEAE 5PW anion-exchange column. Solutions were stored frozen at -70°. [Morosoli et al. *Biochem J* **239** 587 *1986*; Wong et al. *Microbiol Rev* **52** 305 *1988*.]

Zeatin

(*trans*-N^6-[4-hydroxy-3-methylbut-2-en-1-yl]adenine) *[1637-39-4]* **M 219.3, m 207-208, 209-209.5°, pK_1 4.4 (basic), pK_2 9.8 (acidic).** Purified by recrystn from EtOH or H_2O. The UV has λ_{max} at 207 and 275nm (ε 1400 and 14650) in 0.1N aqueous HCl; 212 and 270nm (ε 17050 and 16150) in aqueous buffer pH 7.2; 220 and 276nm (ε 15900 and 14650) in 0.1N aq NaOH. The *picrate* has **m** 192-194° (from H_2O) from which zeatin can be recovered by treatment with Dowex 1 x 8 (200-400 mesh, OH^- form). [Letham et al. *Aust J Chem* **22** 205 *1969*; *Proc Chem Soc (London)* 230 *1964*; Shaw and Wilson *Proc Chem Soc (London)* 231 *1964*.] It is a cell division factor (plant growth regulator) [Letham and Palni *Ann Rev Plant Physiol* **34** 163 *1983*] and inhibits mitochondrial function [Miller *Plant Physiol* **69** 1274 *1982*]. Its *9-riboside* is a cytokine [McDonald and Morris *Methods Enzymol* **100** 347 *1985*].