CHAPTER 6

PURIFICATION OF BIOCHEMICALS AND RELATED PRODUCTS

Biochemicals are chemical substances produced by living organisms. They range widely in size, from simple molecules such as formic acid and glucose to macromolecules such as proteins and nucleic acids. Their *in vitro* synthesis is often impossibly difficult and in such cases they are available (if at all) only as commercial tissue extracts which have been subjected to purification procedures of widely varying stringency. The desired chemical may be, initially, only a minor constituent of the source tissue which may vary considerably in its composition and complexity. Recent advances in molecular biology have made it possible to produce substantial amounts of biological materials, which are present in nature in extremely small amounts, by recombinant DNA technology and expression in bacteria, yeast, insect and mammalian cells. The genes for these substances can be engineered such that the gene products, e.g. polypeptides or proteins, can be readily obtained in very high states of purity. However, many such products which are still obtained from the original natural sources are available commercially and may require further purification.

As a preliminary step the tissue might be separated into phases [e.g. whole egg into white and yolk, blood into plasma (or serum) and red cells], and the desired phase may be homogenised. Subsequent treatment usually comprises filtration, solvent extraction, salt fractionation, ultracentrifugation, chromatographic purification, gel filtration and dialysis. Fractional precipitation with ammonium sulfate gives crude protein species. Purification is finally judged by the formation of a single band of macromolecule (e.g. protein) on electrophoresis and/or analytical ultracentrifugation. Although these generally provide good evidence of high purity, none-the-less it does not follow that one band under one set of experimental conditions is an absolute indication of homogeneity.

During the past 20 or 30 years a wide range of methods for purifying substances of biological origin have become available. For small molecules (including many sugars and amino acids) reference should be made to Chapters 1 and 2. The more important methods used for large molecules, polypeptides and proteins in particular, comprise:

1. *Centrifugation.* In addition to centrifugation for sedimenting proteins after ammonium sulfate precipitation in dilute aqueous buffer, this technique has been used for fractionation of large molecules in a denser medium or a medium of varying density. By layering sugar solutions of increasing densities in a centrifuge tube, proteins can be separated in a sugar-density gradient by centrifugation. Smaller DNA molecules (e.g. plasmid DNA) can be separated from RNA or nuclear DNA by centrifugation in aqueous cesium chloride (*ca* 0.975g/mL of buffer) for a long time (e.g. 40h at 40,000 x g). The plasmid DNA band appears at about the middle of the centrifuge tube, and is revealed by the fluorescent pink band formed by the binding of DNA to ethidium bromide which is added to the CsCl buffer. *Microfuges* are routinely used for centrifugation in Eppendorf tubes (1.2-2mL) and can run up to speeds of 12,000 x g. *Analytical centrifugation*, which is performed under specific conditions in an analytical ultracentrifuge is very useful for determining purity, aggregation of protein subunits and the molecular weight of macromolecules. [D.Rickwood, T.C.Ford and J.Steensgaard *Centrifugation: Essential Data Series*, J Wiley & Sons, NY, *1994*].

2. *Gel filtration* with polyacrylamide (mol wt exclusion limit from 3000 to 300,000) and agarose gel (mol wt exclusion limit 0.5 to 150 x 10^6) is useful for separating macromolecules. In this technique high-molecular weight substances are too large to fit into the gel microapertures and pass rapidly through the matrix (with the void volume), whereas low molecular weight species enter these apertures and are held there for longer periods of time, being retarded by the column material in the equilibria, relative to the larger molecules. This method is also used for desalting solutions of macromolecules. *Dry gels* and *crushed beads* are also

useful in the gel filtration process. Selective retention of water and inorganic salts by the gels or beads (e.g. Sephadex G-25) results in increased concentration and purity of the protein fraction which moves with the void volume. (See also Chapter 1, pp 23, 41).

3. *Ion exchange matrices* are microreticular polymers containing carboxylic acid (e.g. Bio-Rad 70) or phosphoric acid (Pharmacia, Amersham Biosciences, Mono-P) exchange functional groups for weak acidic cation exchangers, sulfonic acid groups (Dowex 50W) for strong acidic cation exchangers, diethylaminoethyl (DEAE) groups for weakly basic anion exchangers and quaternary ammonium (QEAE) groups for strong anion exchangers. The old cellulose matrices for ion exchanges have been replaced by Sephadex, Sepharose or Fractogel which have more even particle sizes with faster and more reproducible flow rates. Some can be obtained in fine, medium or coarse grades depending on particle size. These have been used extensively for the fractionation of peptides, proteins and enzymes. The use of *p*H buffers controls the strength with which the large molecules are bound to the support in the chromatographic process. Careful standardisation of experimental conditions and similarly the very uniform size distribution of Mono beads has led to high resolution in the purification of protein solutions. MonoQ (Pharmacia, Amersham Biosciences) is a useful strong anion exchanger, and MonoS (Pharmacia, Amersham Biosciences) is a useful strong cation exchanger whereas MonoP is a weak cation exchanger. These have been successful with medium pressure column chromatography (FPLC, see below in 8). Chelex 100 binds strongly and removes metal ions from macromolecules. [See also Chapter 1, pp. 22-24.]

4. *Hydroxylapatite* is used for the later stages of purification of enzymes. It consists essentially of hydrated calcium phosphate which has been precipitated in a specific manner. It combines the characteristics of gel and ionic chromatography. Crystalline hydroxylapatite is a structurally organised, highly polar material which, in aqueous solution (in buffers) strongly adsorbs macromolecules such as proteins and nucleic acids, permitting their separation by virtue of the interaction with charged phosphate groups and calcium ions, as well as by physical adsorption. The procedure therefore is not entirely ion-exchange in nature. Chromatographic separations of singly and doubly stranded DNA are readily achievable whereas there is negligible adsorption of low molecular weight species.

5. *Affinity chromatography* is a chromatographic technique whereby the adsorbant has a particular and specific affinity for one of the components of the mixture to be purified. For example the adsorbant can be prepared by chemically binding an inhibitor of a specific enzyme (which is present in the crude complex mixture) to a matrix (e.g. Sepharose). When the mixture of impure enzyme is passed through the column containing the adsorbant, only the specific enzyme binds to the column. After adequate washing, the pure enzyme can be released from the column by either increasing the salt concentration (e.g. NaCl) in the eluting buffer or adding the inhibitor to the eluting buffer. The salt or inhibitor can then be removed by dialysis, gel filtration (above) or ultrafiltration (see below). [See W.H.Scouten, *Affinity Chromatography: Bioselective Adsorption on Inert Matrices*, J.Wiley & Sons, NY, *1981*, ISBN 0471026492; H.Schott, *Affinity Chromatography: Template Chromatography of Nucleic Acids and Proteins*, Marcel Dekker, NY, 1984, ISBN 0824771117; P.Matejtschuk ed. *Affinity Separations* Oxford University Press 1997 ISBN 0199635501 (paperback); M.A.Vijayalakshmi, *Biochromatography, Theory and Practice*, Taylot & Francis Publ, 2002, ISBN 0415269032; and Chapter 1, p. 25.]

6. In the *Isoelectric focusing* of large charged molecules on polyacrylamide or agarose gels, slabs of these are prepared in buffer mixtures (e.g. ampholines) which have various pH ranges. When a voltage is applied for some time the buffers arrange themselves on the slabs in respective areas according to their pH ranges (prefocusing). Then the macromolecules are applied near the middle of the slab and allowed to migrate in the electric field until they reach the pH area similar to their isoelectric points and focus at that position. This technique can also be used in a chromatographic mode, *chromatofocusing*, whereby a gel in a column is run (also under HPLC conditions) in the presence of ampholines (narrow or wide pH ranges as required) and the macromolecules are then run through in a buffer. *Capillary electrophoresis* systems in which a current is applied to set the gradient are now available in which the columns are fine capillaries and are used for qualitative and quantitative purposes [See R.Kuhn and S.Hoffstetter-Kuhn, *Capillary Electrophoresis*: Principles and Practice, Springer-Verlag Inc, NY, *1993*; P.Camilleri ed. *Capillary Electrophoresis - Theory and Practice*, CRC Press, Boca Raton, Florida, *1993*; D.R.Baker, *Capillary Electrophoresis*, J Wiley & Sons, NY, *1995*; P.G.Righetti, A.Stoyanov and M.Zhukov, *The Proteome Revisited, Isoelectric Focusing; J.Chromatography Library Vol* **63** 2001, Elsevier, ISBN 0444505261.] The bands are eluted according to their isoelectric points. Isoelectric focusing standards are available which can be used in a preliminary run in order to calibrate the effluent from the column, or alternatively the pH of the effluent is recorded using a glass electrode designed for the purpose. Several efficient commercially available apparatus are available for separating proteins on a preparative and semi-preparative scale.

7. *High performance liquid chromatography* (HPLC) is liquid chromatography in which the eluting liquid is sent through the column containing the packing (materials as in 2-6 above, which can withstand higher than atmospheric pressures) under pressure. On a routine basis this has been found useful for purifying

proteins (including enzymes) and polypeptides after enzymic digestion of proteins or chemical cleavage (e.g. with CNBr) prior to sequencing (using reverse-phase columns such as μ-Bondapak C18). Moderate pressures (50-300psi) have been found most satisfactory for large molecules (FPLC). [See Scopes *Anal Biochem* **114** 8 *1981*; *High Performance Liquid Chromatography and Its Application to Protein Chemistry,* Hearn in *Advances in Chromatography,* **20** 7 *1982*; B. A. Bidlingmeyer *Practical HPLC Methodology and Applications*, J Wiley & Sons, NY *1991*; L.R.Snyder, J.L.GlajCh and J.J.Kirkland *Practical HPLC Method Development*, J Wiley & Sons, NY *1988*; ISBN 0471627828; R.W.A.Oliver, *HPLC of Macromolecules: A Practical Approach*, 2nd Edn, Oxford University Press, *1998*, T.Hanai, *HPLC: A Practical Guide*, Royal Society of Chemistry (UK), *1999*, ISBN 084045155; P.Millner *High Resolution Chromatography*, Oxford University Press, *1999* ISBN 0199636486; see also Chapter 1, bibliography.]

8. *Ultrafiltration* using a filter (e.g. Millipore) can remove water and low-molecular weight substances without the application of heat. Filters with a variety of molecular weight exclusion limits not only allow the concentration of a particular macromolecule to be determined, but also the removal (by washing during filtration) of smaller molecular weight contaminants (e.g. salts, inhibitors or cofactors). This procedure has been useful for changing the buffer in which the macromolecule is present (e.g. from Tris-Cl to ammonium carbonate), and for desalting. Ultrafiltration can be carried out in a stirrer cell (Amicon) in which the buffer containing the macromolecule (particularly protein) is pressed through the filter, with stirring, under argon or nitrogen pressure (e.g. 20-60psi). During this filtration process the buffer can be changed. This is rapid (e.g. 2L of solution can be concentrated to a few mLs in 1 to 2h depending on pressure and filter). A similar application uses a filter in a specially designed tube (Centricon tubes, Amicon) and the filtration occurs under centrifugal force in a centrifuge (4-6000rpm at 0°/40min). The macromolecule (usually DNA) then rests on the filter and can be washed on the filter by centrifugation. The macromolecule is recovered by inverting the filter, placing a conical receiver tube on the same side where the macromolecule rests, filling the other side of the filter tube with eluting solution (usually a very small volume e.g. 100 μL), and during further centrifugation this solution passes through the filter and collects the macromolecule from the underside into the conical receiver tube.

9. *Partial precipitation* of a protein in solution can often be achieved by controlled addition of a strong salt solution, e.g ammonium sulfate. This is commonly the first step in the purification process. Its simplicity is offset by possible denaturation of the desired protein and the (sometimes gross) contamination with other proteins. It should therefore be carried out by careful addition of small aliquots of the powdered salt or concentrated solution (below 4°, with gentle stirring) and allowing the salt to be evenly distributed in the solution before adding another small aliquot. Under carefully controlled conditions and using almost pure protein it is sometimes possible to obtain the protein in crystalline form suitable for X-ray analysis (see below).

10. *Dialysis.* This is a process by which small molecules, e.g. ammonium sulfate, sodium chloride, are removed from a solution containing the protein or DNA using a membrane which is porous to small molecules. The solution (e.g. 10mL) is placed in a dialysis bag or tube tied at both ends, and stirred in a large excess of dialysing solution (e.g. 1.5 to 2 L), usually a weak buffer at *ca* 4°. The dialysing buffer is replaced with fresh buffer several times, e.g. four times in 24h. This procedure is similar to ultrafiltration (above) and allows the replacement of buffer in which the protein, or DNA, is dissolved. It is also possible to concentrate the solutions by placing the dialysis tube or bag in Sephadex G25 which allows the passage of water and salts from the inside of the bag thus concentrating the protein (or DNA) solution. Dialysis tubing is available from various distibutors but "Spectra/por" tubing (from Spectrum Medical Industries, Inc, LA) is particularly effective because it retains macromolecules and allows small molecules to dialyse out very rapidly thus reducing dialysing time considerably. This procedure is used when the buffer has to be changed so as to be compatible with the next purification or storage step, e.g. when the protein (or DNA) needs to be stored frozen in a particular buffer for extended periods.

11. *Gel Electrophoresis.* This is becoming a more commonly used procedure for purifying proteins, nucleic acids, nucleoproteins, polysaccharides and carbohydrates. The gels can be electroblotted onto membranes and the modern procedures of identifying, sequencing (proteins and nucleic acids) and amplifying (nucleic acids) on sub-micro scales have made this technique of separation a very important one. See below for polyacrylamide gel electrophoresis (PAGE), [D.Patel *Gel Electrophoresis*, J.Wiley-Liss, Inc., 1994; P.Jones and D.Rickwood, *Gel Electrophoresis: Nucleic Acids* , J.Wiley and Sons,1999 (paperback) ISBN 0471960438; D.M.Gersten and D.Gersten, *Gel Electrophoresis: Proteins*, J.Wiley and Sons Inc, 1996 ISBN 0471962651; R.Westermeier *Electrophoresis in Practice*, 3rd Edn, Wiley-VCH, NY, 2001, ISBN 3527303006].

12. *Crystallisation.* The ultimate in purification of proteins or nucleic acids is crystallisation. This involves very specialised procedures and techniques and is best left to the experts in the field of X-ray crystallography who provide a complete picture of the structure of these large molecules. [A. Ducruix and R. Giegé eds, *Crystallisation of Nucleic Acids and Proteins: A Practical Approach*, 2nd Edition, 2000,

Oxford University Press, ISBN 0199636788 (paperback); T.L.Blundell and L.N.Johnson *Protein Crystallisation*, Academic Press, NY, 1976; A.McPherson *Preparation and Analysis of Protein Crystals*, J.Wiley & Sons, NY, 1982; A.McPherson, *Crystallisation of Biological Macromolecules*, Cold Spring Harbour Laboratory Press, 2001 ISBN 0879696176.]

Other details of the above will be found in Chapters 1 and 2 which also contain relevant references.

Several illustrations of the usefulness of the above methods are given in the *Methods Enzymol* series (Academic Press) in which 1000-fold purifications or more, have been readily achieved. In applying these sensitive methods to macromolecules, reagent purity is essential. It is disconcerting, therefore, to find that some commercial samples of the widely used affinity chromatography ligand Cibacron Blue F3GA contained this dye only as a minor constituent. The major component appeared to be the dichlorotriazinyl precursor of this dye. Commercial samples of Procion Blue and Procion Blue MX-R were also highly heterogeneous [Hanggi and Cadd *Anal Biochem* **149** 91 *1985*]. Variations in composition of sample dyes can well account for differences in results reported by different workers. The purity of substances of biological origin should therefore be checked by one or more of the methods given above. Water of high purity should be used in all operations. Double glass distilled water or water purified by a MilliQ filtration system (see Chapter 2) is most satisfactory.

Brief general procedures for the purification of polypeptides and proteins. Polypeptides of up to *ca* 1-2000 (10-20 amino acid residues) are best purified by reverse phase HPLC. The desired fractions that are collected are either precipitated from solution with EtOH or lyophilised. The purity can be checked by HPLC and identified by microsequencing (1-30 picomoles) to ascertain that the correct polypeptide was in hand. Polypeptides larger than these are sometimes classified as proteins, and are purified by one or more of the procedures described above. The purification of enzymes and functional proteins which can be identified by specific interactions is generally easier to follow because enzyme activities or specific protein interactions can be checked (by assaying) after each purification step. The commonly used procedures for purifying soluble proteins involve the isolation of an aqueous extract from homogenised tissues or extracts from ruptured cells from microorganisms or specifically cultured cells, for example, by sonication, freeze shocking or passage through a small orifice under pressure. Contaminating nucleic acids are removed by precipitation with a basic protein, e.g. protamine sulfate. The soluble supernatant is then subjected to fractionation with increasing concentrations of ammonium sulfate. The required fractions are then further purified by the procedures described in sections 2-9 above. If an affinity adsorbant has been identified then affinity chromatography can provide an almost pure protein in one step sometimes even from the crude extract. The rule of thumb is that a solution with a protein concentration of 1mg/mL has an absorbance A_{1cm} at 280nm of 1.0 units. Membrane-bound proteins are usually insoluble in water or dilute aqueous buffer and are obtained from the insoluble fractions, e.g. the microsomal fractions from the >100,000 x g ultracentrifugation supernatant. These are solubilised in appropriate detergents, e.g. Mega-10 (nonionic), Triton X-100 (ionic) detergents, and purified by methods 2 to 8 (previous section) in the presence of detergent in the buffer used. They are assayed also in the presence of detergent or membrane lipids.

The purity of proteins is best checked by *polyacrylamide gel electrophoresis* (PAGE). The gels are either made or purchased as pre-cast gels and can be with uniform or gradient gel composition. Proteins are applied onto the gels *via* wells set into the gels or by means of a comb, and travel along the gel surface by means of the current applied to the gel. When the buffer used contains sodium dodecylsulfate (SDS) the proteins are denatured and the denatured proteins (e.g. as protein subunits) separate on the gels mainly according to their molecular sizes. These can be identified by running marker proteins, with a range of molecular weights, simultaneously on a track alongside the proteins under study. The protein bands are visualised by fixing the gel (20% acetic acid) and staining with Coomassie blue followed by silver staining if higher sensitivity is required. An Amersham-Pharmacia "Phast Gel Electrophoresis" apparatus is very useful for rapid analysis of proteins. It uses small pre-cast polyacrylamide gels (two gels can be run simultaneously) with various uniform or gradient polyacrylamide concentrations as well as gels for isoelectric focusing. The gels are usually run for 0.5-1h and can be stained and developed (1-1.5h) in the same apparatus. The equipment can be used to electroblot the protein bands onto a membrane from which the proteins can be isolated and sequenced or subjected to antibody or other identification procedures. It should be noted that all purification procedures are almost always carried out at *ca* 4° in order to avoid denaturation or inactivation of the protein being investigated. Anyone contemplating the purification of a protein is referred to: Professor R.K.Scopes's monograph *Protein Purification*, 3rd edn, Springer-Verlag, New

York, *1994*, ISBN 0387940723; M.L.Ladisch ed. *Protein Purification - from Molecular Mechanisms to Large-scale Processes*, American Chemical Society, Washington DC, *1990*; E.L.V.Harris and S.Angal, *Protein Purification Applications - A Practical Approach*, IRL Press, Oxford, *1990*; J.C.Janson and L.Rydén, *Protein Purification - Principles, High Resolution Methods and Applications*, VCH Publ. Inc., *1989*; ISBN 0895731223 R.Burgess, *Protein Purification - Micro to Macro*, A.R.Liss, Inc., NY, *1987*; S.M.Wheelwright, *Protein Purification: Design and Scale up of Downstream Processing*, J Wiley & Sons, NY, *1994*, references in the bibliography in Chapter 1, and selected volumes of *Methods Enzymol*, e.g. M.P.Deutscher ed. Guide to Protein Purification, *Methods Enzymol*, Academic Press, NY, Vol 182 *1990*, ISBN 0121820831; T.Palzkill, *Proteomics*, Kluwer Academic Publ, *2001*, ISBN 0792375653; M.A.Vijayalakshmi, *Biochromatography, Theory and Practice*, Taylot & Francis Publ, *2002*, ISBN 0415269032; J.S.Davies, *Amino Acids, Peptides and Proteins Vol* **32** *2001*, A Specialist Periodical Report, Royal Society of Chemistry, ISBN 0854042326; S.Roe, *Protein Purification Techniques: A Practical Approach*, 2nd Edn, Oxford University Press, *2001*, ISBN 0199636737; T.Palmer, *Enzymes, Biochemistry, Biotechnology, Clinical Chemistry*, Horwood Publishing, *2001*, ISBN 1898563780.

Brief general procedures for purifying DNA. Oligo-deoxyribonucleotides (up to *ca* 60-mers) are conveniently purified by HPLC (e.g. using a Bio-Rad MA7Q anion exchange column and a Rainin Instrument Co, Madison, Dynamax-300A C_8 matrix column) and used for a variety of molecular biology experiments. Plasmid and chromosomal DNA can be isolated by centrifugation in caesium chloride buffer (see section 1. centrifugation above), and then re-precipitated with 70% ethanol at -70° (18h), collected by centrifugation (microfuge) and dried in air before dissolving in TE (10mM TrisHCl, 1mM EDTA pH 8.0). The DNA is identified on an Agarose gel slab (0.5 to 1.0% DNA grade in 45mM Tris-borate + 1mM EDTA or 40mM Tris-acetate + 1mM EDTA pH 8.0 buffers) containing ethidium bromide which binds to the DNA and under UV light causes it be visualised as pink fluorescent bands. Marker DNA (from λ phage DNA cut with the restriction enzymes Hind III and/or EcoRI) are in a parallel track in order to estimate the size of the unknown DNA. The DNA can be isolated from their band on the gel by transfer onto a nitro-acetate paper (e.g. NA 45) electrophoretically, by binding to silica or an ion exchange resin, extracted from these adsorbents and precipitated with ethanol. The DNA pellet is then dissolved in TE buffer and its concentration determined. A solution of duplex DNA (or RNA) of 50μg/mL gives an absorbance of 1.0units at 260nm/1cm cuvette (single stranded DNA or RNA gives a value of 1.3 absorbance units). DNA obtained in this way is suitable for molecular cloning. For experimental details on the isolation, purification and manipulation of DNA and RNA the reader is referred to: J.Sambrook, E.F.Fritsch and T.Maniatis, *Molecular Cloning - A Laboratory Manual* , 2nd edn, (3 volumes), Cold Spring Harbor Laboratory Press, NY, *1989*, ISBN 0879693096 (paperback); P.D.Darbre, *Basic Molecular Biology: Essential Techniques*, J.Wiley and Sons, *1998*, ISBN 0471977055; J.Sambrook and D.W.Russell, *Molecular Cloning - A Laboratory Manual* , 3rd edn, (3 volumes), Cold Spring Harbor Laboratory Press, NY, *2001*, ISBN 0079695773 (paperback), ISBN 0079695765 (cloth bound), also available on line; M.A.Vijayalakshmi, *Biochromatography, Theory and Practice*, Taylot & Francis Publ, *2002*, ISBN 0415269032; A.Travers and M.Buckle, *DNA-Protein Interactions: A Practical Approach*, Oxford University Press, *2000*, ISBN 0199636915 (paperback); R.Rapley and D.L.Manning eds *RNA: Isolation and Characterisation Protocols*, Humana Press *1998* ISBN 086034941; R.Rapley, *The Nucleic Acid Protocols Handbook*, Humana Press 2000 ISBN 0896038416 (paperback).

This chapter lists some representative examples of biochemicals and their origins, a brief indication of key techniques used in their purification, and literature references where further details may be found. Simpler low molecular weight compounds, particularly those that may have been prepared by chemical syntheses, e.g. acetic acid, glycine, will be found in Chapter 4. Only a small number of enzymes and proteins are included because of space limitations. The purification of some of the ones that have been included has been described only briefly. The reader is referred to comprehensive texts such as the *Methods Enzymol* (Academic Press) series which currently runs to more than 344 volumes and *The Enzymes* (3rd Edn, Academic Press) which runs to 22 volumes for methods of preparation and purification of proteins and enzymes. Leading references on proteins will be found in *Advances in Protein Chemistry* (59 volumes, Academic Press) and on enzymes will be found in *Advances in Enzymology* (72 volumes, then became *Advances in Enzymology and Related Area of Molecular Biology*, J Wiley & Sons). The *Annual Review of Biochemistry* (Annual Review Inc. Patlo Alto California) also is an excellent source of key references to the up-to-date information on known and new natural compounds, from small molecules, e.g. enzyme cofactors to proteins and nucleic acids.

Abbreviations of titles of periodical are defined as in the Chemical Abstracts Service Source Index (CASSI).

Ionisation constants of ionisable compounds are given as **pK** values (published from the literature) and refer to the **pKa** values at room temperature (~ 15°C to 25°C). The values at other temperatures are given as superscripts, e.g. $\mathbf{pK}^{25}$ for 25°C. Estimated values are entered as $\mathbf{pK}_{Est(1)}$~ (see Chapter 1, p 6 for further information).

Benzene, which has been used as a solvent successfully and extensively in the past for reactions and purification by chromatography and crystallisation is now considered a **very dangerous substance** so it has to be used with extreme care. We emphasise that an alternative solvent to benzene (e.g. toluene, toluene-petroleum ether, or a petroleum ether to name a few) should be used first. However, if benzene has to be used then all operations have to be performed in a well ventilated fumehood and precautions taken to avoid inhalation and contact with skin and eyes. Whenever benzene is mentioned in the text and asterisk e.g. $^*C_6H_6$ or *benzene, is inserted to remind the user that special precaution should be adopted.

Amino acids, **carbohydrates** and **steroids** not found below are in Chapter 4 (see also CAS Registry Numbers Index and General Index).

Abrin A and Abrin B *[1393-62-0]* $\mathbf{M_r}$ **63,000-67,000.** Toxic proteins from seeds of *Abras precatorius.* Purified by successive chromatography on DEAE-Sephadex A-50, carboxymethylcellulose, and DEAE-cellulose. [Wei et al. *J Biol Chem* **249** 3061 *1974.*]

Acetoacetyl coenzyme A trisodium salt trihydrate *[102029-52-7]* **M 955.6, pK_1 4.0 (NH_2), pK_2 6.4 (PO_4^-).** The pH of solution (0.05g/mL H_2O) is adjusted to 5 with 2N NaOH. This solution can be stored frozen for several weeks. Further purification can be carried out on a DEAE-cellulose formate column, then through a Dowex 50 (H^+) column to remove Na ions, concentrated by lyophilisation and redissolved in H_2O. Available as a soln of 0.05g/mL of H_2O. The concn of acetoacetylcoenzyme A is determined by the method of Stern et al. *J Biol Chem* **221** 15 *1956.* It is stable at pH 7-7.5 for several hours at 0° (half life *ca* 1-2h). At room temperature it is hydrolysed in *ca* 1-2h at pH 7-7.5. At pH 1.0/20° it is more stable than at neutrality. It is stable at pH 2-3/-17° for at least 6 months. [*J Biol Chem* **159** 1961 *1964*; **242** 3468 *1967*; Clikenbeard et al. *J Biol Chem* **250** 3108 *1975*; *J Am Chem Soc* **75** 2520 *1953,* **81** 1265 *1959*; see Simon and Shemin *J Am Chem Soc* **75** 2520 *1953*; Salem et al. *Biochem J* **258** 563 *1989.*]

Acetobromo-α-D-galactose *[3068-32-4]* **M 411.2, m 87°, $[\alpha]_{546}^{20}$ +255°, $[\alpha]_D^{20}$ +210° (c 3, $CHCl_3$).** Purified as for the glucose analogue (see next entry). If the compound melts lower than 87° or is highly coloured then dissolve in $CHCl_3$ (*ca* 3 vols) and extract with H_2O (2 vols), 5% aqueous $NaHCO_3$, and again with H_2O and dry over Na_2SO_4. Filter and evaporate in a vacuum. The partially crystalline solid or syrup is dissolved in dry Et_2O (must be very dry) and recrystd by adding pet ether (b 40-60°) to give a white product. [McKellan and Horecker *Biochem Prep* **11** 111 *1960.*]

Acetobromo-α-D-glucose *[572-09-8]* **M 411.2, m 87-88°, 88-89°, $[\alpha]_{546}^{20}$ +230°, $[\alpha]_D^{20}$ +195° (c 3, $CHCl_3$).** If nicely crystalline recryst from Et_2O-pentane. Alternatively dissolve in diisopropyl ether (dried over $CaCl_2$ for 24hours, then over P_2O_5 for 24hours) by shaking and warming (for as short a period as possible), filter warm. Cool to *ca* 45° then slowly to room temperature and finally at 5° for more than 2hours. Collect the solid, wash with cold dry diisopropyl ether and dry in a vacuum over $Ca(OH)_2$ and NaOH. Store dry in a desiccator in the dark. Solutions can be stabilised with 2% $CaCO_3$. [Redemann and Niemann *Org Synth* **65** 236 *1987*, Coll Vol III 11 *1955.*]

Acetoin dehydrogenase [from beef liver; acetoin NAD oxidoreductase] *[9028-49-3]* $\mathbf{M_r}$ **76000, [EC 1.1.1.5].** Purified *via* the acetone cake then Ca-phosphate gel filtration (unabsorbed), lyophilised and then fractionated through a DEAE-22 cellulose column. The Km for diacetyl in 40μM and for

NADH it is 100μM in phosphate buffer at pH 6.1. [Burgos and Martin *Biochim Biophys Acta* **268** 261 *1972*; **289** 13 *1972*.]

(-)-3-β-Acetoxy-5-etienic acid [3-β-acetoxy-5-etiocholenic acid, androst-5-ene-17-β-carboxylic acid] *[51424-66-9]* **M 306.5, m 238-240°, 241-242°, 243-245°, 246-247°, $[\alpha]_D^{20}$ -19.9° (c 1, Me_2CO), -36° (c 1, Dioxane), -33.5° ($CHCl_3$), pK_{Est} ~ 4.7.** It is purified by recrystn from Me_2CO, Et_2O-pentane, or AcOH, and dried in a vacuum oven (105°/20mm) and sublimed at high vacuum. [Staunton and Eisenbram *Org Synth* **42** 4 *1962*; Steiger and Reichstein *Helv Chim Acta* **20** 1404 *1937*.]

Acetylcarnitine chloride (2-acetoxy-3-carboxy-*N,N,N*-trimethylpropanamine HCl) *[S(D+)-5080-50-2; R(L-)- 5061-35-8; RS 2504-11-2]* **M 239.7, m 181°, 197°(dec), $[\alpha]_D^{25}$ -28° (c 2, H_2O) for *S*-isomer, pK^{25} 3.6.** Recrystd from isopropanol. Dried over P_2O_5 under high vacuum.

Acetylcholine bromide *[66-23-9]* **M 226.1, m 143°, 146°.** *Hygroscopic* solid but less than the hydrochloride salt. It crystd from EtOH as prisms. Some hydrolysis occurs in boiling EtOH particularly if it contains some H_2O. It can also be recryst from EtOH or MeOH by adding dry Et_2O. [*Acta Chem Scand* **12** 1492, 1497, 1502 *1958*.]

Acetylcholine chloride *[60-31-1]* **M 181.7, m 148-150°, 151°.** It is very sol in H_2O (> 10%), and is very *hygroscopic*. If pasty, dry in a vacuum desiccator over H_2SO_4 until a solid residue is obtained. Dissolve in abs EtOH, filter and add dry Et_2O and the hydrochloride separates. Collect by filtration and store under very dry conditions. [*J Am Chem Soc* **52** 310 *1930*.] The *chloroplatinate* crystallises from hot H_2O in yellow needles and can be recrystd from 50% EtOH, **m** 242-244° [*Biochem J* **23** 1069 *1929*], other **m** given is 256-257°. The *perchlorate* crystallises from EtOH as prisms **m** 116-117°. [*J Am Pharm Assocn* **36** 272 *1947*.]

***N^4*-Acetylcytosine** *[14631-20-0]* **M 153.1, m >300°, 326-328°, $pK_{Est(1)}$ ~1.7, $pK_{Est(2)}$ ~10.0.** If TLC or paper chromatography show that it contains unacetylated cytosine then reflux in Ac_2O for 4h, cool at 3-4° for a few days, collect the crystals, wash with cold H_2O, then EtOH and dry at 100°. It is insoluble in EtOH and difficulty soluble in H_2O but crystallises in prisms from hot H_2O. It is hydrolysed by 80% aq AcOH at 100°/1h. [*Am Chem J* **29** 500 *1903*; UV: *J Chem Soc* 2384 *1956*; *J Am Chem Soc* **80** 5164 *1958*.] It forms an Hg salt [*J Am Chem Soc* **79** 5060 *1957*].

β-D-*N*-Acetylglucosaminidase [from M sexta insects] *[9012-33-3]* **M_r ~61,000, [EC 3.2.1.52].** Purified by chromatography on DEAD-Biogel, hydroxylapatite chromatography and gel filtration through Sephacryl S200. Two isoforms: a hexosaminidase EI with Km 177μM (V_{max} 328 sec^{-1}) and EII a chitinase with Km 160μM (V_{max} 103 sec^{-1}) with 4-nitrophenyl-β-acetylglucosamine as substrate. [Dziadil-Turner *Arch Biochem Biophys* **212** 546 *1981*.]

β-D-*N*-Acetylhexosaminidase A and B (from human placenta) *[9012-33-3]* **M_r ~61,000, [EC 3.2.1.52].** Purified by Sephadex G-200 filtration and DEAE-cellulose column chromatography. Hexosaminidase A was further purified by DEAE-cellulose column chromatography, followed by an ECTEOLA-cellulose column, Sephadex-200 filtration, electrofocusing and Sephadex G-200 filtration. Hexosaminidase B was purified by a CM-cellulose column, electrofocusing and Sephadex G-200 filtration. [Srivastava et al. *J Biol Chem* **249** 2034 *1974*.]

***N*-Acetyl-D-lactosamine [2-acetylamino-*O*-β-D-lactopyranosyl-2-deoxy-D-glucose]** *[32181-59-2]* **M 383.4, m 169-171°, 170-171°, $[\alpha]_D^{18}$ +51.5°→ +28.8° (in 3h, c 1, H_2O].** Purified by recrystn from MeOH (with 1 mol of MeOH) or from H_2O. It is available as a soln of 0.5g /mL of H_2O. [Zilliken *J Biol Chem* **271** 181 *1955*.]

***O*-Acetyl-β-methylcholine chloride [Methacholine chloride, Amechol, Provocholine, 2-acetoxypropyl-ammonium chloride]** *[62-51-1]* **M 195.7, m 170-173°, 172-173°.** It forms white *hygroscopic* needles from Et_2O and is soluble in H_2O, EtOH and $CHCl_3$. It decomposes readily in alkaline solns and slowly in H_2O. It should be handled and stored in a dry atmosphere. The *bromide* is less

hygroscopic and the *picrate* has **m** 129.5-131° (from EtOH). [racemate: Annis and Ely *Biochem J* **53** 34 *1953*; IR of iodide: Hansen *Acta Chem Scand* **13** 155 *1959*.]

***N*-Acetyl muramic acid [NAMA, *R*-2-(acetylamino)-3-*O*-(1-carboxyethyl)-2-deoxy-D-glucose]** *[10597-89-4]* **M 292.3, m ~125°(dec), $[\alpha]_D^{20}$ +41.2° (c 1.5, H_2O, after 24h), pK_{Est} ~ 3.6.** See muramic acid below.

***N*-Acetyl neuraminic acid (NANA, *O*-Sialic acid, 5-acetamido-3,5-dideoxy-D-*glycero*-D-*glacto*-2-nonulosonic acid, lactaminic acid)** *[131-48-6]* **M 309.3, m 159°(dec), 181-183°(dec), 185-187°(dec), $[\alpha]_D^{25}$ -33° (c 2, H_2O, l 2), pK 2.6.** A Dowex-1x8 (200-400 mesh) in the formate form was used, and was prepd by washing with 0.1M NaOH, then 2N sodium formate, excess formate was removed by washing with H_2O. *N*-Acetyl neuraminic acid in H_2O is applied to this column, washed with H_2O, then eluted with 2N formic acid at a flow rate of 1mL/min. Fractions (20mL) were collected and tested (Bial's orcinol reagent, *cf Biochem Prep* **7** 1 *1959*). NANA eluted at formic acid molarity of 0.38 and the Bial positive fractions are collected and lyophilised. The residue is recrystd from aqueous AcOH: Suspend 1.35g of residue in AcOH, heat rapidly to boiling, add H_2O dropwise until the suspension dissolves (do not add excess H_2O, filter hot and then keep at +5° for several hours until crystn is complete. Collect and dry in a vacuum over P_2O_5. Alternatively dissolve 1.35g of NANA in 14mL of H_2O, filter, add 160mL of MeOH followed by 360mL of Et_2O. Then add pet ether (b 40-60°) until heavy turbidity. Cool at 20° overnight. Yield of NANA is *ca* 1.3g. Dry over P_2O_5 at 1mm vacuum and 100° to constant weight. It mutarotates in Me_2SO: $[\alpha]_D^{20}$ -115° (after 7min) to -32° (after 24h). It is available as aqueous soln (0.01g/mL). [IR and synthesis: Cornforth et al. *Biochem J* **68** 57 *1958*; Zillikin and O'Brien *Biochem Prep* **7** 1 *1960*; ^{13}C NMR and 1-^{13}C synthesis: Nguyen, Perry *J Org Chem* **43** 551 *1978*; Danishevski, DeNinno *J Org Chem* **51** 2615 *1986*; Gottschalk, *The Chemistry and Biology of Sialic Acids and Related Substances*, Cambridge University Press, London, *1960*.]

***N*-Acetyl neuraminic acid aldolase [from *Clostridium perfringens*, *N*-acetylneuraminic acid pyruvate lyase]** *[9027-60-5]* **M_r 32,000 [EC 4.1.3.3].** Purified by extraction with H_2O, protamine pptn, $(NH_4)_2SO_4$ pptn, Me_2CO pptn, acid treatment at pH 5.7 and pptn at pH 4.5. The equilibrium constant for pyruvate + *n*-acetyl-D-mannosamine ⇌ *N*-acetylneuraminidate at 37° is 0.64. The Km for *N*-acetylneuraminic acid is 3.9mM in phosphate at pH 7.2 and 37°. [Comb and Roseman *Methods Enzymol* **5** 391 *1962*.] The enzyme from Hogg kidney (cortex) has been purified 1700 fold by extraction with H_2O, protamine sulfate pptn, $(NH_4)_2SO_4$ pptn, heating between 60-80°, a second $(NH_4)_2SO_4$ pptn and starch gel electrophoresis. The Km for *N*-acetylneuraminic acid is 1.5mM. [Brunetti et al. *J Biol Chem* **237** 2447 *1962*.]

***N*-Acetyl penicillamine** *[D- 15537-71-0, DL-59-53-0]* **M 191.3, m 183°, 186-187° (DL-form), 189-190° (D-form), D-form $[\alpha]_D^{25}$ +18° (c 1, 50% EtOH), $pK_{Est(1)}$~3.0 (CO_2H), $pK_{Est(2)}$~ 8.0 (SH).** Both forms are recrystd from hot H_2O. A pure sample of the D-form was obtained after five recrystns. [Crooks in *The Chemistry of Penicillin* Clarke, Johnson and Robinson eds, Princeton University Press, 470 *1949*.]

***p*-Acetylphenyl sulfate potassium salt,** *[38533-41-4]* **M 254.3, m dec on heating, pK_{Est} ~2,1.** Purified by dissolving in the minimum vol of hot water (60°) and adding EtOH, with stirring, then left at 0° for 1h. Crystals were filtd off and recrystd from H_2O until free of Cl^- and SO_4^{2-} ions. Dried in a vac over P_2O_5 at room temperature. It is a specific substrate for arylsulfatases which hydrolyse it to *p*-acetylphenol [λmax 327nm (ε 21700 $M^{-1}cm^{-1}$)] [Milsom et al. *Biochem J* **128** 331 *1972*].

S-Acetylthiocholine bromide *[25025-59-6]* **M 242.2, m 217-223°(dec).** It is a *hygroscopic* solid which can be recrystd from ligroin-EtOH (1:1), dried and kept in a vacuum desiccator. Crystn from $^*C_6H_6$-EtOH gave **m** 227° or from propan-1-ol the **m** was 213°. [*Acta Chem Scand* **11** 537 *1957*, **12** 1481 *1958*.]

S-Acetylthiocholine chloride *[6050-81-3]* **M 197.7, m 172-173°** The chloride can be purified in the same way as the bromide, and it can be prepared from the iodide. A few milligrams dissolved in H_2O can be purified by applying onto a Dowex-1 Cl^- resin column (prepared by washing with N HCl followed by CO_3^{2-}—free H_2O until the pH is 5.8). After equilibration for 10min elution is started with CO_3^{2-}—free distilled H_2O and

3mL fractions are collected and their OD at 229nm measured. The fractions with appreciable absorption are pooled and lyophilised at 0-5°. Note that at higher temps decomposition of the ester is appreciable; hydrolysis is appreciable at pH >10.5/20°. The residue is dried *in vacuo* over P_2O_5, checked for traces of iodine (conc H_2SO_4 and heat, violet vapours are released), and recrystd from propan-1-ol. [*Clin Chim Acta* **2** 316 *1957*.]

S-Acetylthiocholine iodide *[1866-15-5]* **M 289.2, m 203-204°, 204°, 204-205°.** Recrystd from propan-1-ol (or *iso*-PrOH, or EtOH/Et_2O) until almost colourless and dried in a vacuum desiccator over P_2O_5. Solubility in H_2O is 1% w/v. A 0.075M (21.7mg/mL) solution in 0.1M phosphate buffer pH 8.0 is stable for 10-15 days if kept refrigerated. Store away from light. It is available as a 1% soln in H_2O. [*Biochemical Pharmacology* **7**, 88 *1961*; IR: Hansen *Acta Chem Scand* **13** 151 *1959*, **11** 537 *1957* ; *Clin Chim Acta* **2** 316 *1957*; *Zh Obshch Khim* **22** 267 *1952*.]

Actinomycin C (Cactinomycin) *[8052-16-2]* **M ~1255.** (A commercial mixture of Actinomycin C_1 ~5%, C_2 ~30% and C_3 ~65%). *Actinimycin C_1 (native)* crysts from EtOAc as red crystals, is sol in $CHCl_3$, $^*C_6H_6$ and Me_2CO and has **m** 246-247°(dec), $[\alpha]_D^{20}$ -328° (0.22, MeOH) and λ_{max} 443nm (ε 25,000) and 240nm (ε 34,000). *Actinimycin C_2 (native)* crysts as red needles from EtOAc and has **m** 244-246°(dec), $[\alpha]_D^{20}$ -325° (c 0.2, MeOH), λ_{max} 443nm (ε 25,300) and (ε 33,400). *Actinimycin C_3 (native)* recryst from cyclohexane, or $^*C_6H_6$/MeOH/cyclohexane as red needles **m** 238-241° (dec), $[\alpha]_D^{20}$ -321° (c 0.2, MeOH), λ_{max} 443nm (ε 25,000) and 240nm (ε 33,300). [Brockman and Lackner, *Chem Ber* **101** 1312 *1968*.] It is *light sensitive*.

Actinomycin D (Dactinomycin) *[50-76-0]* **M 1255.5, m 241-243°(dec), $[\alpha]_D^{22}$ -296° (c 0.22, MeOH).** Crystallises as bright red rhombic crystals from absolute EtOH or from MeOH-EtOH (1:3). It will also crystallise from EtOAc-cyclohexane (**m** 246-247° dec), $CHCl_3$-pet ether (**m** 245-246° dec), and EtOAc-MeOH-$^*C_6H_6$ (**m** 241-243° dec). Its solubility in MeCN is 1mg/mL. $[\alpha]_D^{20}$ varies from -296° to -327° (c 0.2, MeOH). λ_{max} (MeOH) 445, 240nm (log ε 4.43, 4.49), λ_{max} (MeOH, 10N HCl, 1:1) 477nm (log ε 4.21) and λ_{max} (MeOH, 0.1N NaOH) 458, 344, 285 (log ε 3.05, 4.28, 4.13). It is *HIGHLY* **TOXIC,** light sensitive and antineoplastic. [Bullock and Johnson, *J Chem Soc* 3280 *1957*.]

Acyl-coenzyme A Synthase [from beef liver] *[9013-18-7]* **M_r 57,000, [EC 6.2.1.2].** Purified by extraction with sucrose-HCO_3 buffer, protamine sulfate pptn, $(NH_4)_2SO_4$ (66-65%) pptn at pH 4.35 and a second $(NH_4)_2SO_4$ (35-60%) pptn at pH 4.35. It has Km 0.15mM (V_{rel} 1.0) for octanoate; 0.41mM (V_{rel} 2.37) for heptanoate and 1.59mM (V_{rel} 0.63). Km for ATP is 0.5mM all at pH 9.0 in ethylene glycol buffer at 38°. [Jencks et al. *J Biol Chem* **204** 453 *1953; Methods Enzymol* **5** 467 *1962*.]

Acyl-coenzyme A Synthase (from yeast) *[9012-31-1]* **[EC 6.2.1.1].** This enzyme has been purified by extraction into phosphate buffer pH 6.8-7.0 containing 2-mercaptoethanol and EDTA, protamine sulfate pptn, polyethylene glycol fractionation, Alumina γ gel filtration, concentration by $(NH_4)_2SO_4$ pptn, Bio-Gel A-0.5m chromatography and DEAE-cellulose gradient chromatography. It has M_r ~151,000, Km (apparent) 0.24mM (for acetate) and 0.035mM (for CoA); 1.2 mM (for ATP) and Mg^{2+} 4.0mM. [Frenkel and Kitchens *Methods Enzymol* **71** 317 *1981*.]

Adenosine-5'-diphosphate [adenosine-5'-pyrophosphate, ADP] *[58-64-0]* **M 427.2, $[\alpha]_D^{25}$ -25.7° (c 2, H_2O), pK_1^{25}<2 (PO_4H), pK_2^{25}<2 (PO_4H), pK_3^{25} 3.95 (NH_2), pK_4^{25} 6.26 (PO_4H).** Characterised by conversion to the *acridine salt* by addition of alcoholic acridine (1.1g in 50mL), filtering off the yellow salt and recrystallising from H_2O. The salt has **m** 215°(dec), λ_{max} 259nm (ε 15,400) in H_2O. [Baddiley and Todd *J Chem Soc* 648 *1947*, 582 *1949*, *cf* LePage *Biochem Prep* **1** 1 *1949*; Martell and Schwarzenbach *Helv Chim Acta* **39** 653 *1956*].

Adenosine-3'-monophosphoric acid hydrate [3'-adenylic acid, 3'-AMP] *[84-21-9]* **M 347.3, m 197°(dec, as $2H_2O$), 210°(dec), m 210°(dec), $[\alpha]_{546}$ -50° (c 0.5, 0.5M Na_2HPO_4), pK_1^{25} 3.65, pK_2^{25} 6.05.** It crystallises from large volumes of H_2O in needles as the monohydrate, but is not very soluble in boiling H_2O. Under acidic conditions it forms an equilibrium mixture of 2' and 3' adenylic acids *via* the 2',3'-cyclic phosphate. When heated with 20% HCl it gives a quantitative yield of furfural after 3hours, unlike 5'-adenylic acid which only gives traces of furfural. The yellow *monoacridine salt* has **m** 175°(dec) and

the *diacridine salt* has **m** 177° (225°)(dec). [Brown and Todd *J Chem Soc* 44 *1952*; Takaku et al. *Chem Pharm Bull Jpn* **21** 1844 *1973*; NMR: Ts'O et al. *Biochemistry* **8** 997 *1969*.]

Adenosine-5'-monophosphoric acid monohydrate [5'-adenylic acid, 5'-AMP] *[18422-05-4]* **M 365.2, m 178°, 196-200°, 200° (sintering at 181°), $[\alpha]_D^{20}$ -47.5°, $[\alpha]_{546}$ -56° (c 2, in 2% NaOH), -26.0° (c 2, 10% HCl), -38° (c 1, 0.5M Na_2HPO_4), pK_1^{25} 3.89, pK_2^{25} 6.14, pK_3^{25} 13.1.** It has been recrystd from H_2O (fine needles) and is freely soluble in boiling H_2O. Crysts also from H_2O by addition of acetone. Purified by chromatography on Dowex 1 (in formate form), eluting with 0.25M formic acid. It was then adsorbed onto charcoal (which had been boiled for 15min with M HCl, washed free of chloride and dried at 100°), and recovered by stirring three times with isoamyl alcohol/H_2O (1:9 v/v). The aqueous layer from the combined extracts was evaporated to dryness under reduced pressure, and the product was crystallised twice from hot H_2O. [Morrison and Doherty *Biochem J* **79** 433 *1961*]. It has λ_{max} 259nm (ε 15,400) in H_2O at pH 7.0. [Alberty et al. *J Biol Chem* **193** 425 *1951*; Martell and Schwarzenbach *Helv Chim Acta* **39** 653 *1956*]. The *acridinium salt* has **m** 208° [Baddiley and Todd *J Chem Soc* 648 *1947*; Pettit *Synthetic Nucleotides,* van Nostrand-Reinhold, NY, **Vol 1** 252 *1972*; NMR: Sarma et al. *J Am Chem Soc* **96** 7337 *1974*; Norton et al. *J Am Chem Soc* **98** 1007 *1976*; IR of *diNa salt*: Miles *Biochem Biophys Acta* **27** 324 *1958*].

Adenosine 5"-[β-thio]diphosphate tri-lithium salt *[73536-95-5]* **M 461.1.** Purified by ion-exchange chromatography on DEAE-Sephadex A-25 using gradient elution with 0.1-0.5M triethylammonium bicarbonate. [*Biochem Biophys Acta* **276** 155 *1972*.]

Adenosine 5"-[α-thio]monophosphate di-lithium salt *[19341-57-2]* **M 375.2.** Purified as for the diNa salt [Murray and Atkinson *Biochemistry* **7** 4023 *1968*]. Dissolve 0.3g in dry MeOH (7mL) and M LiI (6mL) in dry Me_2CO containing 1% of mercaptoethanol and the Li salt is ppted by adding Me_2CO (75mL). The residue is washed with Me_2CO (4 x 30mL) and dried at 55°/25mm. λ_{max} (HCl, pH 1.2) 257nm (ε 14,800); (0.015M NaOAc, pH 4.8) 259nm (ε 14,800); and (0.015M NH_4OH, pH 10.1) 259nm (ε 15,300).

Adenosine-5'-triphosphate (ATP) *[56-65-5]* **M 507.2, $[\alpha]_{546}$ -35.5 (c 1, 0.5 M Na_2HPO_4), pK_1^{25} 4.00, pK_2^{25} 6.48.** Ppted as its barium salt when excess barium acetate soln was added to a 5% soln of ATP in water. After filtering off, the ppte was washed with distd water, redissolved in 0.2M HNO_3, and again pptd with barium acetate. The ppte, after several washings with distd water, was dissolved in 0.2M HNO_3 and slightly more 0.2M H_2SO_4 than was needed to ppte all the barium as $BaSO_4$, was added. After filtering off the $BaSO_4$, the ATP was ppted by addition of a large excess of 95% ethanol, filtered off, washed several times with 100% EtOH and finally with dry diethyl ether. [Kashiwagi and Rabinovitch *J Phys Chem* **59** 498 *1955*.]

S-(5'-Adenosyl)-L-homosysteine *[979-92-0]* **M 384.4, m 202°(dec), 204°(dec), 205-207°(dec), $[\alpha]_D^{25}$ +93° (c 1, 0.2N HCl), $[\alpha]_D^{23}$ +44° (c 0.1, 0.05N HCl), (pK see SAM hydrochloride below).** It has been recrystd several times from aqueous EtOH or H_2O to give small prisms and has λ_{max} 260nm in H_2O. The *picrate* has **m** 170°(dec) from H_2O. [Baddiley and Jameison *J Chem Soc* 1085 *1955*; de la Haba and Cantoni *J Biol Chem* **234** 603 *1959*; Borchardt et al. *J Org Chem* **41** 565 *1976*; NMR: Follmann et al. *Eur J Biochem* **47** 187 *1974*.]

(-)-S-Adenosyl-L-methionine chloride (SAM hydrochloride) *[24346-00-7]* **M 439.9, $pK_{Est(1)}$~ 2.13, $pK_{Est(2)}$~ 4.12, $pK_{Est(3)}$~ 9.28.** Purified by ion exchange on Amberlite IRC-150, and eluting with 0.1-4M HCl. [Stolowitz and Minch *J Am Chem Soc* **103** 6015 *1981*.] It has been isolated as the tri-reineckate salt by adding 2 volumes of 1% solution of ammonium reineckate in 2% perchloric acid. The reineckate salt separates at once but is kept at 2° overnight. The salt is collected on a sintered glass funnel, washed with 0.5% of ammonium reineckate, dried (all operations at 2°) and stored at 2°. To obtain adenosylmethionine, the reineckate is dissolved in a small volume of methyl ethyl ketone and centrifuged at room temp to remove a small amount of solid. The clear dark red supernatant is extracted (in a separating funnel) with a slight excess of 0.1 N H_2SO_4. The aqueous phase is re-extracted with fresh methyl ethyl ketone until it is colourless. [Note that reineckates have UV absorption at 305nm (ε 15,000), and the optical density at 305nm is used to detect the presence of reineckate ions.] Methyl ethyl ketone is removed from the aqueous layer containing adenosylmethionine sulfate, the pH is adjusted to 5.6-6.0 and extracted with two volumes of Et_2O.

The *sulfate* is obtained by evaporating the aqueous layer in *vacuo*. The *hydrochloride* can be obtained in the same way but using HCl instead of H_2SO_4. SAM-HCl has a solubility of 10% in H_2O. The salts are stable in the cold at pH 4-6 but decompose in alkaline media. [Cantoni *Biochem Prep* **5** 58 *1957*.] The purity of SAM can be determined by paper chromatography [Cantoni *J Biol Chem* **204** 403 *1953*; *Methods Enzymol* **3** 601 *1957*], and electrophoretic methods or enzymic analysis [Cantoni and Vignos *J Biol Chem* **209** 647 *1954*].

L-Adrenaline [L-epinephrine, L(-)-(3,4-dihydroxyphenyl)-2-methylaminoethanol] *[51-43-4]* **M 183.2, m 210°(dec), 211°(dec), 211-212°(dec), 215°(dec), $[\alpha]_{D}^{20}$ -52° (c 2, 5% HCl), pK_1^{25} 8.88, pK_2^{25} 9.90, pK_3^{25} 12.0.** It has been recrystd from EtOH + AcOH + NH_3 [Jensen *J Am Chem Soc* **57** 1765 *1935*]. It is sparingly soluble in H_2O, readily in acidic or basic solns but insoluble in aqueous NH_3, alkali carbonate solns, EtOH, $CHCl_3$, Et_2O or Me_2CO. It is readily oxidised in air and turns brown on exposure to light and air. Store in the dark under N_2. Its pKa values in H_2O are 8.88 and 9.90 [Lewis *Br J Pharmacol Chemother* **9** 488 *1954*]. The *hydrogen oxalate salt* has **m** 191-192°(dec, evac capillary) after recrystn from H_2O or EtOH [Pickholz *J Chem Soc* 928 *1945*].

Adrenolone hydrochloride [3',4'-dihydroxy-2-methylaminoacetophenone hydrochloride] *[62-13-5]* **M 217.7, m 244-249°(dec), 248°(dec), 256°(dec), pK 5.5.** It was purified by recrystn from EtOH or aqueous EtOH. [Gero *J Org Chem* **16** 1222 *1951*; Kindler and Peschke *Arch Pharm* **269** 581, 603 *1931*.]

ADP-Ribosyl transferase (from human placenta) *[9026-30-6]*. Purified by making an affinity absorbent for ADP-ribosyltransferase by coupling 3-aminobenzamide to Sepharose 4B. [Burtscher et al. *Anal Biochem* **152** 285 *1986*.]

Agglutinin (from peanuts) [*Arachis hypogaea*] *[1393-62-0]* **M_r 134,900 (tetramer).** Purified by affinity chromatography on Sepharose-ζ-aminocaproyl-ß-D-galactopyranosylamine. [Lotan et al. *J Biol Chem* **250** 8518 *1974*.]

Alamethicin (from *Tricoderma viridae*). *[27061-78-5]* **M 1964.3, m 259-260°, 275-270°, $[\alpha]_D^{22}$ -45° (c 1.2, EtOH), pK 6.04 (aq EtOH).** Recrystd from MeOH. [Panday et al. *J Am Chem Soc* **99** 8469 *1977*.] The *acetate [64918-47-4]* has **m** 195-180° from MeOH/Et_2O and the *acetate-methyl ester [64936-53-4]* has **m** 145-140° from aq MeOH.

Albumin (bovine and human serum) *[9048-46-8 (bovine); 70024-90-7 (human)]* **M_r ~67,000 (bovine), 69 000 (human), UV: $A_{280nm}^{1\%}$ 6.6 (bovine) and 5.3 (human) in H_2O, $[\alpha]_{546}^{25}$ -78.2° (H_2O).** Purified by soln in conductivity water and passage at 2-4° through two ion-exchange columns, each containing a 2:1 mixture of anionic and cationic resins (Amberlite IR-120, H-form; Amberlite IRA-400, OH-form). This treatment removed ions and lipid impurities. Care was taken to exclude CO_2, and the soln was stored at -15°. [Möller, van Os and Overbeek *Trans Faraday Soc* **57** 312 *1961*.] More complete lipid removal was achieved by lyophilising the de-ionised soln, covering the dried albumin (human serum) with a mixture of 5% glacial acetic acid (v/v) in iso-octane (previously dried with Na_2SO_4) and allowing to stand at 0° (without agitation) for upwards of 6h before decanting and discarding the extraction mixture, washing with iso-octane, re-extracting, and finally washing twice with iso-octane. The purified albumin was dried under vacuum for several hours, then dialyzed against water for 12-24h at room temperature, lyophilised, and stored at -10°C [Goodman *Science* **125** 1296 *1957*]. It has be recrystd in high (35%) and in low (22%) EtOH solutions from Cohn's Fraction V.

The **high EtOH recrystn** was as follows: To 1 Kg of Fraction V albumin paste at -5° was added 300mL of 0.4 M pH (pH 5.5) acetate buffer in 35% EtOH pre-cooled to -10° and 430 mL of 0.1 M NaOAc in 25% EtOH also at -10°. Best results were obtained by adding all of the buffer and about half of the NaOAc and stirring slowly for 1hour. The rest of the NaOAc was added when all the lumps had disintegrated. The mixture was set aside at -5° for several days to crystallise. 35% EtOH (1 L) was then added to dilute the crystalline suspension and lower the ionic strength prior to centrifugation at -5° (yield 80%). The crystals were further dissolved in 1.5 volumes of 15% EtOH-0.02M NaCl at -5° and clarified by filtration through washed, calcined diatomaceous earth. This soln may be recrystd by re-adjusting to the conditions in the first crystallisation, or it may be recrystd at 22% EtOH with the aid of a very small amount of decanol (enough to give a final concn of 0.02%).

Note that crystn from lower EtOH gave better purification (i.e. by removing globulins and carbohydrates) and producing a more stable product.
The **low EtOH recrystn** was as follows: To 1 Kg of Fraction V at -10° to -15° was added 500mL of 15% EtOH at -5°, stirred slowly until a uniform suspension was formed. 15% EtOH (500mL) and sufficient 0.2M $NaHCO_3$ soln at 0° to bring the pH (1:10 diln) to 5.3. This required 125- 150mL . Some temp rise occurs and care must be taken to keep the temp < -5°. If the albumin is incompletely dissolved a small amount of H_2O was added (100mL at a time at 0°, allowing 15min between additions). Undissolved albumin can be easily distinguished from small amounts of undissolved globulins, or as the last albumin dissolves, the appearance of the soln changes from milky white to hazy grey-green in colour. Keep the soln at -5° for 12h and filter by suspending in 15g of washed fine calcined diatomaceous earth, and thus filtering using a Büchner funnel precoated with coarser diatomaceous earth. The filtrate may require two or more similar filtrations to give a clear soln. To crystallise the filtrate add through a capillary pipette, and with careful stirring, 1/100volume of a soln containing10% decanol and 60% EtOH (at -10°), and seeded with the needle-type albumin crystals. After 2-3 days crystn is complete. The crystals are centrifuged off. These are suspended with gentle mechanical stirring in one third their weight of 0.005 M NaCl pre-cooled to 0°. With careful stirring, H_2O (at 0°) is added slowly in an amount equal to 1.7 times the weight of the crystals. At this stage there is about 7% EtOH and the temp cannot be made lower than -2.5° to -1°. Clarify and collect as above. [Cohn et al. *J Am Chem Soc* **69** 1753 *1947*.]
Human serum albumin has been purified similarly with 25% EtOH and 0.2% decanol. The isoelectric points of bovine and human serum albumins are 5.1 and 4.9.

Amethopterin (Methotrexate, 4-amino-4-deoxy-N^{10}-methylpteroyl-L-glutamic acid) *[59-05-2]* **M 454.4, m 185-204°(dec), $[\alpha]_D^{20}$ +19° (c 2, 0.1N aq NaOH), pK_1 <0.5 (pyrimidine^{2+}), pK_2 2.5 (N5-Me$^+$), pK_3 3.49 (α-CO_2H), pK_4 4.99 (γ-CO_2H), pK_5 5.50 (pyrimidine$^+$).** Commonest impurities are 10-methyl pteroylglutamic acid, 4-amino-10-methylpteroylglutamic acid, aminopterin and pteroylglutamic acid. Purified by chromatography on Dowex-1 acetate, followed by filtration through a mixture of cellulose and charcoal. It has been recrystd from aqueous HCl or by dissolution in the minimum volume of N NaOH and acidified until pptn is complete, filter or collect by centrifugation, wash with H_2O (also by centrifugation) and dry at 100°/3mm. It has UV λ_{max} at 244 and 307nm (ε 17300 and 19700) in H_2O at pH 1; 257, 302 and 370nm (ε 23000, 22000 and 7100) in H_2O at pH 13. [Momle *Biochem Prep* **8** 20 *1961*; Seeger et al. *J Am Chem Soc* **71** 1753 *1949*.] It is a potent inhibitor of dihydrofolate reductase and used in cancer chemotherapy. [Blakley *The Biochemistry of Folic Acid and Related Pteridines* (North-Holland Publ Co., Amsterdam, NY) pp157-163 *1969*.] It is **CARCINOGENIC, HANDLE WITH EXTREME CARE.**

α-Amino acids. All the α-amino acids 'natural' configuration [*S* (L), except for cysteine which is *R*(L)] at the α- carbon atom are available commercially in a very high state of purity. Many of the 'non-natural' α-amino acids with the [*R*(D)] configuration as well as racemic mixtures are also available and generally none require further purification before use unless they are of "Technical Grade'. The *R* or *S* enantiomers are optically active except for glycine which has two hydrogen atoms on the α- carbon atom, but these are *pro*-chiral and enzymes or proteins do distinguish between them, e.g. serine hydroxymethyltransferase successfully replaces the *pro*-α- hydrogen atom of glycine with CH_2OH (from formaldehyde) to make *S*-serine. The twenty common natural α-amino acids are: **amino acid**, three letter abbreviation, **one letter abbreviation**, pK (-COOH) and pK (-NH_3^+): **Alanine**, Ala, **A**, 2.34, 9.69; **Arginine**, Arg, **R**, 2.17, 9.04; **Asparagine**, Asn, **N**, 2.01, 8.80; **Aspartic acid**, Asp, **D**, 1.89, 9.60; **Cysteine**, Cys, **C**, 1.96, 8.18; **Glutamine**, Gln, **Q**, 2.17, 9.13; **Glutamic acid**, Glu, **E**, 2.19, 9.67; **Glycine**, Gly, **G**, 2.34, 9.60; **Histidine**, His, **H**, 1.8, 9.17; **Isoleucine**, Ile, **I**, 2.35, 9.68; **Leucine**, Leu, **L**, 2.36, 9.60; **Lysine**, Lys, **K**, 2.18, 8.95; **Methionine**, Met, **M**, 2.28, 9.20; **Phenylalanine**, Phe, **F**, 1.83, 9.12; **Proline**, Pro, **P**, 1.99, 10.96; **Serine**, Ser, **S**, 2.21, 9.15; **Threonine**, Thr, **T**, 2.11, 9.62; **Tryptophan**, Trp, **W**, 2.38, 9.39; **Tyrosine**, Tyr, **Y**, 2.2, 9.11, **Valine**, Val, **V**, 2.32, 9.61 repectively. Technical grade amino acids can be purified on ion exchange resins (e.g. Dowex 50W and eluting with a gradient of HCl or AcOH) and the purity is checked by TLC in two dimensions and stained with ninhydrin. (J.P.Greenstein and M.Winitz, *Chemistry of the Amino Acids* (3 Volumes), J.Wiley & Sons, NY, 1961; C.Cooper, N.Packer and K.Williams, *Amino Acid Analysis Protocols*, Humana Press, 2001, ISBN 0896036561). Recently codons for a further two amino acids have been discovered which are involved in ribosome-mediated protein synthesis giving proteins containing these amino

acids. The amino acids are *R*(L)-selenocysteine [Stadtman *Ann Rev Biochem* **65** 83 *1996*] and pyrrolysine [(4*R*, 5*R*)-4-substituted (with Me, NH_2 or OH) pyrroline-5-carboxylic acid] [Krzychi and Chan et al. *Science* **296** 1459 and 1462 *2002*.] They are, however, rare at present and only found in a few microorganisms.

9-Aminoacridine hydrochloride monohydrate (Acramine yellow, Monacrin) *[52417-22-8]* **M 248.7, m >355°, pK_1^{20} 4.7, pK_2^{20} 9.99.** Recrystd from boiling H_2O (charcoal; 1g in 300 mL) to give pale yellow crystals with a neutral reaction. It is one of the most fluorescent substances known. At 1:1000 dilution in H_2O it is pale yellow with only a faint fluorescence but at 1:100,000 dilution it is colourless with an intense blue fluorescence. [Albert and Ritchie *Org Synth* Coll Vol III 53 *1955*; Falk and Thomas *Pharm J* **153** 158 *1944*.] See entry in Chapter 4 for the free base.

Aminopterin (4-amino-4-deoxypteroyl-L-glutamic acid) *[54-62-6]* **M 440.4, m 231-235°(dec), $[\alpha]_D^{20}$ +18° (c 2, 0.1N aq NaOH), pK_1 <0.5 (pyrimidine^{2+}), pK_2 2.5 (N5-Me$^+$), pK_3 3.49 (α-CO_2H), pK_4 4.65 (γ-CO_2H), pK_5 5.50 (pyrimidine$^+$).** Purified by recrystn from H_2O, and has properties similar to those of methotrexate. It has UV at λ_{max} 244, 290 and 355nm (ε 18600, 21300 and 12000) in H_2O at pH 1; 260, 284 and 370nm (ε 28500, 26400 and 8600) in H_2O at pH 13. [Seeger et al. *J Am Chem Soc* **71** 1753 *1949*; Angier and Curran *J Am Chem Soc* **81** 2814 *1959*; Blakley *The Biochemistry of Folic Acid and Related Pteridines* (North-Holland Publ Co., Amsterdam, NY) pp157-163 *1969*.] For small quantities chromatography on DEAE cellulose with a linear gradient of ammonium bicarbonate pH 8 and increasing the molarity from 0.1 to 0.4 and followed by UV is best. For larger quantities a near boiling solution of aminopterin (5g) in H_2O (400mL) was slowly treated with small portions of MgO powder (~0.7g) calcined magnesia) with vigorous stirring until a small amount of MgO remained undissolved and the pH rises from 3-4 to 7-8. Charcoal (1g) is added to the hot solution, filtered at once through a large sintered glass funnel of medium porosity and lined with a hot wet pad of Celite (~2-3 mm thick). The filtrate is cooled in ice and the crystals of the Mg salt are collected by filtration and recrystd form boiling H_2O (200mL) and the crystals washed with EtOH. The Mg salt is redissolved in boiling H_2O (200mL) and carefully acidified with vigorous agitation with AcOH (2mL). Pure aminopterin (3g) separates in fine yellow needles (dihydrate) which are easily filtd. The filtrate is washed with cold H_2O, then Me_2CO and dried in vac. If a trace of impurity is still present as shown by DEAE cellulose chromatography, then repetition of the process will remove it, see UV above. [Loo *J Med Chem* **8** 139 *1965*.] **CARCINOGENIC**

3-Aminopyridine adenine dinucleotide *[21106-96-7]* **M 635.4 (see NAD for pK)** Purified by ion exchange chromatography [Fisher et al. *J Biol Chem* **248** 4293 *1973*; Anderson and Fisher *Methods Enzymol* **66** 81 *1980*].

α–Amino-thiophene-2-acetic acid 2-(2-thienyl)glycine *[R(+)- 65058-23-3; S(-)- 4052-59-9; (-)- 43189-45-3; RS(±)- 21124-40-3]* **M 57.2, m 236-237° (*R*), 189-191°, 235-236° (*S*), 208-210°, 223-224° (dec)(*RS*), $[\alpha]_D^{20}$ (+) and (-) 84° (c 1, 1% aq HCl), $[\alpha]_D^{25}$ (+) and (-) 71° (c 1 H_2O), $pK_{Est(1)}$~ 1.5, $pK_{Est(2)}$~ 8.0.** Recrystd by dissolving in H_2O (1g in 3 mL), adjusting the pH to 5.5 with aq NH_3, diluting with MeOH (20 mL), stirring, adjusting the pH to 5.5 and cooling to 0°. Also recrystd from small vols of H_2O. [*R*-isomer: Nishimura et al. *Nippon Kagaku Zasshi* **82** 1688 *1961; S*-isomer: Johnson and Panetta *Chem Abstr* **63** 14869h *1965*; Johnson and Hardcastle *Chem Abstr* **66** 10930m *1967*; *RS*-isomer:LiBassi et al. *Gazz Chim Ital* **107** 253 *1977*.] The *(±) N-acetyl* derivative has **m** 191° (from H_2O) [Schouteenten et al. *Bull Soc Chim Fr* II-248, II-252 *1978*].

4(6)-Aminouracil (4-amino-2,6-dihydroxypyrimidine) *[873-83-6]* **M 127.1, m >350°, pK_1^{20} 0.00 (basic), pK_2^{20} 8.69 (acidic), pK_3^{20} 15.32 (acidic).** Purified by dissolving in 3M aq NH_3, filter hot, and add 3M formic acid until pptn is complete. Cool, filter off (or centrifuge), wash well with cold H_2O, then EtOH and dry in air. Dry further in a vac at ~80°. [Barlin and Pfeiderer *J Chem Soc (B)* 1424 *1971*.]

Amylose *[9005-82-7]* **$(C_6H_{10}O_5)_n$ *(for use in iodine complex formation).*** Amylopectin was removed from impure amylose by dispersing in aqueous 15% pyridine at 80-90° (concn 0.6-0.7%) and leaving the soln stand at 44-45° for 7 days. The ppte was re-dispersed and recrystd during 5 days. After a further dispersion in 15% pyridine, it was cooled to 45°, allowed to stand at this temperature for 12hours, then cooled

to 25° and left for a further 10hours. The combined ppte was dispersed in warm water, ppted with EtOH, washed with absolute EtOH, and vacuum dried [Foster and Paschall *J Am Chem Soc* **75** 1181 *1953*].

Angiotensin (from rat brain) *[70937-97-2]* **M 1524.8.** Purified using extraction, affinity chromatography and HPLC [Hermann et al. *Anal Biochem* **159** 295 *1986*].

Angiotensinogen (from human blood serum) *[64315-16-8].* Purified by chromatography on Blue Sepharose, Phenyl-Sepharose, hydroxylapatite and immobilised 5-hydroxytryptamine [Campbell et al. *Biochem J* **243** 121 *1987*].

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

ß-Apo-4'-carotenal *[12676-20-9]* **M 414.7, m 139°,** $A^{1\%}_{1cm}$ **2640 at 461nm** Recrystd from $CHCl_3$/EtOH mixture or *n*-hexane. [Bobrowski and Das *J Org Chem* **91** 1210 *1987*.]

ß-Apo-8'-carotenal *[1107-26-2]* **M 414.7, m 136-139°.** Recrystd from $CHCl_3$/EtOH mixture or *n*-hexane. [Bobrowski and Das *J Org Chem* **91** 1210 *1987*.]

ß-Apo-8'-carotenoic acid ethyl ester *[1109-11-1]* **M 526.8, m 134-138°,** $A^{1\%}_{1cm}$ **2550 at 449nm.** Crystd from pet ether or pet ether/ethyl acetate. Stored in the dark in an inert atmosphere at -20°.

ß-Apo-8'-carotenoic acid methyl ester *[16266-99-2]* **M 512.7, m 136-137°,** $A^{1\%}_{1cm}$ **2575 at 446nm and 2160 at 471nm, in pet ether.** Crystd from pet ether or pet ether/ethyl acetate. Stored in the dark in an inert atmosphere at -20°.

Apocodeine *[641-36-1]* **M 281.3, m 124°,** $pK_{Est(1)}$**~ 7.0,** $pK_{Est(2)}$**~ 8.2.** Crystd from MeOH and dried at 80°/2mm.

Apomorphine *[58-00-4]* **M 267.3, m 195°(dec),** pK_1^{15} **7.20 (NH_2),** pK_2^{15} **8.91 (phenolic OH).** Crystd from $CHCl_3$ and pet ether, also from Et_2O with 1 mol of Et_2O which it loses at 100°. It is white but turns green in moist air or in alkaline soln. **NARCOTIC**

Apomorphine hydrochloride *[41372-20-7]* **M 312.8, m 285-287°(dec),** $[\alpha]_D^{20}$ **-48° (c 1 H_2O).** Cryst from H_2O and EtOH. Crystals turn green on exposure to light. **NARCOTIC**

Aureomycin (7-chlorotetracycline) *[57-62-5]* **M 478.5, m 172-174°(dec),** $[\alpha]_D^{23}$ **-275° (MeOH),** pK_1 **3.3,** pK_2 **7.44,** pK_3 **9.27.** Dehydrated by azeotropic distn of its soln with toluene. On cooling anhydrous material crystallises out and is recrystd from $^*C_6H_6$, then dried under vacuum at 100° over paraffin wax. (If it is crystd from MeOH, it contains MeOH which is not removed on drying.) [Stephens et al. *J Am Chem Soc* **76** 3568 *1954*; *Biochem Biophys Res Commun* **14** 137 *1964*].

Aureomycin hydrochloride (7-chlorotetracycline hydrochloride) *[64-72-2]* **M 514.0, m 234-236°(dec),** $[\alpha]_D^{25}$ **-23.5° (H_2O).** Purified by dissolving 1g rapidly in 20mL of hot water, cooling rapidly to 40°, treating with 0.1mL of 2M HCl, and chilling in an ice-bath. The process is repeated twice. Also recrystd from Me_2NCHO + Me_2CO. [Stephens et al. *J Am Chem Soc* **76** 3568 *1954* ; UV: McCormick et al. *J Am Chem Soc* **79** 2849 *1975*.]

Avidin (from egg white) *[1405-69-2]* M_r **~70,000.** Purified by chromatography of an ammonium acetate soln on CM-cellulose [Green *Biochem J* **101** 774 *1966*]. Also purified by affinity chromatography on 2-iminobiotin-6-aminohexyl-Sepharose 4B [Orr *J Biol Chem* **256** 761 *1981*]. It is a biotin binding protein.

Azurin (from *Pseudomonas aeruginosa*) *[12284-43-4]* **M_r 30,000.** Material with $A_{625/A280}$ = 0.56 was purified by gel chromatography on G-25 Sephadex with 5mM phosphate pH 7 buffer as eluent [Cho et al. *J Phys Chem* **91** 3690 *1987*]. It is a blue Cu protein used in biological electron transport and its reduced form is obtained by adding a slight excess of $Na_2S_2O_4$. [See *Structure and Bonding* Springer Verlag, Berlin **23** 1 *1975*.]

Bacitracin (Altracin, Topitracin)

[1405-87-4] **M 1422.7, $[\alpha]_D^{23}$ +5° (H_2O).** It has been purified by carrier displacement using *n*-heptanol, *n*-octanol and *n*-nonanol as carriers and 50% EtOH in 0.1 N HCl. The pure material gives one spot with R_F ~0.5 on paper chromatography using AcOH:*n*-BuOH: H_2O (4:1:5). [Porath *Acta Chem Scand* **6** 1237 *1952*.] It has also been purified by ion-exchange chromatography. It is a white powder soluble in H_2O and EtOH but insoluble in Et_2O, $CHCl_3$ and Me_2CO. It is stable in acidic soln but unstable in base. (Abraham and Bewton *Biochem J* **47** 257 *1950*; Synthesis: Munekata et al. *Bull Chem Soc Jpn* **46** 3187, 3835 *1973*.]

N^6-Benzyladenine *[1214-39-7]* **M 225.3, m 231-232°, 232.5°(dec), $pK_{Est(1)}$~ 4.2, $pK_{Est(2)}$~ 10.1.** Purified by recrystn from aqueous EtOH. It has λ_{max} at 207 and 270nm (H_2O), 268 nm (pH 6), 274nm (0.1 N HCl) and 275nm (0.1 N NaOH). [Daly *J Org Chem* **21** 1553 *1956*; Bullock et al. *J Am Chem Soc* **78** 3693 *1956*.]

N^6-Benzyladenosine *[4294-16-0]* **M 357.4, m 177-179°, 185-187°, $[\alpha]_D^{25}$-68.6° (c 0.6, EtOH)(see above entry for pK).** Purified by recrystn from EtOH. It has λ_{max} 266nm (aq EtOH-HCl) and 269 nm (aqueous EtOH-NaOH). [Kissman and Weiss *J Org Chem* **21** 1053 *1956*.]

***N*-Benzylcinchoninium chloride (9*S*-benzyl-9-hydroxycinchoninium chloride)** *[69221-14-3]* **M 421.0, $[\alpha]_D^{20}$ +169° (c 0.4, H_2O), pK_{Est} ~ 5.** Recrystd from isoPrOH, toluene or small volumes of H_2O. Good chiral phase transfer catalyst [Julia et al. *J Chem Soc Perkin Trans 1* 574 *1981*; Hughes et al. *J Am Chem Soc* **106** 446 *1984*; Hughes et al. *J Org Chem* **52** 4745 *1987*]. See cinchonine below.

***R*-(-)-*N*-Benzylcinchonidinium chloride** *[69257-04-1]* **M 421.0, m 212-213° (dec), $[\alpha]_D^{20}$ -175.4°, -183° (c 5, 0.4, H_2O), pK_{Est} ~5.** Dissolve in minimum volume of H_2O and add absolute Me_2CO. Filter off and dry in a vacuum. Also recrystd from hot EtOH or EtOH-Et_2O. (A good chiral phase transfer catalyst - see above) [Colonna et al. *J Chem Soc Perkin Trans 1* 547 *1981*, Imperali and Fisher *J Org Chem* **57** 757 *1992*]. See cinchonidine below.

***N*-Benzylpenicillin sodium salt** *[69-57-8]* **M 356.37, m 215° (charring and dec), 225° (dec), $[\alpha]_D^{20}$ +269° (c 0.7, MeOH), $[\alpha]_D^{25}$ +305° (c 1, H_2O), pK^{25} 2.76 (4.84 in 80% aq EtOH)(for free acid).** Purified by dissolving in a small volume of MeOH (in which it is more soluble than EtOH) and treating gradually with ~5 volumes of EtOAc. This gives an almost colourless crystalline solid (rosettes of clear-cut needles) and recrystallising twice more if slightly yellow in colour. The salt has also been conveniently recrystd from the minimum amount of 90% Me_2CO and adding an excess of absolute Me_2CO. A similar procedure can be used with wet *n*-BuOH. If yellow in colour then dissolve (~3.8g) in the minimum volume of H_2O (3mL), add *n*-BuOH and filter through a bed of charcoal. The salt forms long white needles on standing in a refrigerator overnight. More crystals can be obtained on concentrating the mother liquors *in vacuo* at 40°. A further recrystn (without charcoal) yields practically pure salt. A good preparation has ~600 Units/mg. The presence of H_2O in the solvents increases the solubility considerably. The solubility in mg/100mL at 0° is 6.0 (Me_2CO), 15.0 (Me_2CO + 0.5% H_2O), 31.0 (Me_2CO + 1.0% H_2O), 2.4 (methyl ethyl ketone), 81.0 (*n*-butanol) and 15.0 (dioxane at 14°). Alternatively it is dissolved in H_2O (solubility is 10%), filtered if necessary and ppted by addition of EtOH and dried in a vacuum over P_2O_5. A sample can be kept for 24h at 100° without loss of physiological activity. [IR: *Anal Chem* **19** 620 *1947*; *The Chemistry of Penicillin* [Clarke, Johnson and Robinson eds.] Princeton University Press, Princeton NJ, Chapter V 85 *1949*.]
Other salts, e.g. the **potassium salt** can be prepared from the Na salt by dissolving it (147mg) ice-cold in H_2O acidified to pH 2, extracting with Et_2O (~50mL), wash once with H_2O, and extract with 2mL portions of 0.3% $KHCO_3$ until the pH of the extract rose to ~6.5 (~7 extractns). The combined aqueous extracts are

lyophilised and the white residue is dissolved in *n*-BuOH (1mL, absolute) with the addition of enough H_2O to effect soln. Remove insoluble material by centrifugation and add absolute *n*-BuOH to the supernatant. Crystals should separate on scratching, and after 2.5h in a refrigerator they are collected, washed with absolute *n*-BuOH and EtOAc and dried (yield 51.4mg). The *potassium salt* has **m** 214-217° (dec) (block preincubated at 200°; heating rate of 3°/min) and $[\alpha]_D^{22}$ +285° (c 0.748, H_2O). The *free acid* has **m** 186-187° (MeOH-Me_2CO), 190-191° (H_2O) $[\alpha]_D^{25}$ +522°.

(+)-Bicuculine [*R*-6(5,6,7,8-tetrahydro-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolon-5-yl)-furo-[3,4-*c*]-1,3-benzodioxolo-8(6*H*)-one] *[485-49-4]* **M 367.4, m 177°, 193-195°, 193-197°, 215°, $[\alpha]_D^{20}$ +126° (c 1, $CHCl_3$), $[\alpha]_{546}^{20}$ +159° (c 1, $CHCl_3$), pK 4.84.** Recrystallises from $CHCl_3$-MeOH as plates. Crystals melt at 177° then solidify and re-melt at 193-195° [Manske *Canad J Research* **21B** 13 *1943*]. It is soluble in $CHCl_3$, $^*C_6H_6$, EtOAc but sparingly soluble in EtOH, MeOH and Et_2O. [Stereochem: Blaha et al. *Collect Czech Chem Commun* **29** 2328 *1964*; Snatzke et al. *Tetrahedron* **25** 5059 *1969*; Pharmcol: Curtis et al. *Nature* **266** 1222 *1970*].

L-*erythro*-Biopterin (2-amino-4-hydroxy-6-[{1*R*,2*S*}-1,2-dihydroxypropyl]pteridine) *[22150-76-1]* **M 237.2, m >300°(dec), $[\alpha]_{546}^{20}$ -65° (c 2.0, M HCl), pK_1^{25} 2.23(2.45), pK_2^{25} 7.89(8.05).** Purified by chromatography on Florisil washed thoroughly with 2M HCl, and eluted with 2M HCl. The fractions with the UV-fluorescent band are evapd *in vacuo* and the residue recrystd. Biopterin is best recrystd (90% recovery) by dissolving in 1% aq NH_3 (*ca* 100 parts), and adding this soln dropwise to an equal vol of M aq formic acid at 100° and allowing to cool at 4° overnight. It is dried at 20° to 50°/01mm in the presence of P_2O_5. [Schircks, Bieri and Viscontini *Helv Chim Acta* **60** 211 *1977*; Armarego, Waring and Paal *Aust J Chem* **35** 785 *1982*.] Also crystd from *ca* 50 parts of water or 100 parts of hot 3M aq HCl by adding hot 3M aq NH_3 and cooling. It has UV: λ_{max} at 212, 248 and 321nm (log ε 4.21, 4.09 and 3.94) in H_2O at pH 0.0; 223infl, 235.5, 274.5 and 345nm (log ε 4.07inflexion, 4.10, 4.18 and 3.82) in H_2O at pH 5.0; 221.5, 254.5 and 364nm (log ε 3.92, 4.38 and 3.84) in H_2O at pH 10.0 [Sugimoto and Matsuura *Bull Chem Soc Jpn* **48** 3767 *1875*].

D-(+)-Biotin (vitamin H, hexahydro-2-oxo-1*H*-thieno[3,4-d]imidazole-4-pentanoic acid) *[58-85-5]* **M 244.3, m 229-231°, 230.2°(dec), 230-231°, 232-234°(dec), $[\alpha]_{546}^{20}$ +108°, $[\alpha]_D^{20}$ +91.3° (c 1, 0.1N NaOH), pK_{Est} ~ 4.8.** Crystd from hot water in fine long needles with a solubility of 22 mg/100mL at 25°. Its solubility in 95% EtOH is 80 mg/100 mL at 25°. Its isoelectric point is at pH 3.5. Store solid and solutions under sterile conditions because it is susceptible to mould growth. [Confalone *J Am Chem Soc* **97** 5936 *1975*; Wolf et al. *J Am Chem Soc* **67** 2100 *1945*; Synthesis: Ohuri and Emoto *Tetrahedron Lett* 2765 *1975*; Harris et al. *J Am Chem Soc* **66** 1756 *1944*.] The *(+)-methyl ester* has **m** 166-167° (from MeOH-Et_2O), $[\alpha]_D^{22}$ +57° (c 1, $CHCl_3$) [du Vigneaud et al. *J Biol Chem* **140** 643, 763 *1941*]; the *(+)-S-oxide* has **m** 200-203°, $[\alpha]_D^{20}$ +130° (c 1.2, 0.1N NaOH) [Melville *J Biol Chem* **208** 495 *1954*]; the *SS-dioxide* has **m** 274-275°(dec, 268-270°) and the *SS-dioxide methyl ester* has **m** 239-241° (from MeOH-Et_2O) [Hofmann et al. *J Biol Chem* **141** 207, 213 *1941*.]

D-(+)-Biotin hydrazide *[66640-86-6]* **M 258.4, m 238-240°, 245-247°, $[\alpha]_D^{20}$ +66° (c 1, Me_2NCHO).** Wash the material with H_2O, dry, wash with MeOH then Et_2O, dry, and recrystallise from hot H_2O (clusters of prisms) [Hofmann et al. *J Biol Chem* **144** 513 *1942*].

D-(+)-Biotin *N*-hydroxysuccinimide ester (+-biotin *N*-succinimidyl ester) *[35013-72-0]* **M 342.4, m 210°, 212-214°, $[\alpha]_D^{20}$ +53° (c 1, Me_2NCHO).** Recrystd from refluxing isoPrOH and dried in a vacuum over P_2O_5 + KOH. [Jasiewicz et al. *Exp Cell Biol* **100** 213 *1976*.]

D-(+)-Biotin 4-nitrophenyl ester *[33755-53-2]* **M 365.4, m 160-163°, 163-165°, $[\alpha]_D^{25}$ +47° (c 2, Me_2NCHO containing 1% AcOH).** It has been recrystd by dissolving 2g in 95% EtOH (30mL), heated to dissolve, then cooled in an ice-water bath. The crystals are collected, washed with ice-cold 95% EtOH (5mL) and dried over P_2O_5. The R_F on silica plates ($CHCl_3$:MeOH-19:1) is 0.19 [Bodanszky and Fagan *J Am Chem Soc* **99** 235 *1977*].

***N*-(+)-Biotinyl-4-aminobenzoic acid** *[6929-40-4]* **M 363.4, m 295-297°, 295-300°, $[\alpha]_D^{23}$ +56.55° (c 0.5, 0.1N NaOH), pK_{Est} ~4.0.** Dissolve in $NaHCO_3$ soln, cool and ppte by adding N HCl. Collect the solid, dry at 100° and recrystallise from MeOH. Note that it is hydrolysed by aq 3M, 1M and 0.2M HCl at 120°, but can be stored in 5% aq $NaHCO_3$ at -20° without appreciable hydrolysis [Knappe et al. *Biochem Zeitschrift* **338** 599 *1963*; *J Am Chem Soc* **73** 4142 *1951*; Bayer and Wilchek *Methods Enzymol* **26** 1 *1980*]

***N*-Biotinyl-6-aminocaproic *N*-succinimidyl ester** *[72040-63-2]* **M 454.5, m 149-152°.** Dissolve ~400mg in dry propan-2-ol (~25mL) with gentle heating. Reduce the volume to ~10mL by gentle boiling and allow the soln to cool. Decant the supernatant carefully from the white crystals, dry the crystals in a vacuum over P_2O_5 at 60° overnight. Material gives one spot on TLC. [Costello et al. *Clin Chem* **25** 1572 *1979*; Kincaid et al. *Methods Enzymol* **159** 619 *1988*.]

***N*-(+)-Biotinyl-6-aminocaproyl hydrazide (biotin-6-aminohexanoic hydrazide)** *[109276-34-8]* **M 371.5, m 189-191°, 210°, $[\alpha]_D^{20}$ +23° (c 1, Me_2NCHO).** Suspend in ice-water (100mg/mL), allow to stand overnight at 4°, filter and dry the solid in a vacuum. Recrystd from isoPrOH. R_F 0.26 on SiO_2 plate using $CHCl_3$-MeOH (7:3) as eluent. [O'Shannessy et al. *Anal Biochem* **163** 204 *1987*.]

***N*-(+)-Biotinyl-L-lysine (Biocytin)** *[576-19-2]* **M 372.5, m 228.5°, 228-230° (dec), 241-243°, 245-252° (dec, sintering at 227°), $[\alpha]_D^{25}$ +53° (c 1.05, 0.1 N NaOH).** Recrystd rapidly from dilute MeOH or Me_2CO. Also recrystd from H_2O by slow evaporation or by dissolving in the minimum volume of H_2O and adding Me_2CO until solid separates. It is freely soluble in H_2O and AcOH but insoluble in Me_2CO. [Wolf et al. *J Am Chem Soc* **76** 2002 *1952*, **72** 1048 *1050*.] It has been purified by chromatography on superfiltrol-Celite, Al_2O_3 and by countercurrent distribution and then recrystd [IR: Peck et al. *J Am Chem Soc* **74** 1991 *1952*]. The *hydrochloride* can be recrystd from aqueous Me_2CO + HCl and has **m** 227° (dec).

2-(4-Biphenylyl)-5-phenyl-1,3,4-oxadiazole *[852-38-0]* **M 298.4, m 166-167°, 167-170°.** Recrystd from toluene. It is a good scintillation material [Brown et al. *Discussion Faraday Soc* **27** 43 *1959*].

2,5-Bis(4-biphenylyl)-1,3,4-oxadiazole (BBOD) *[2043-06-3]* **M 374.5, m 229-230°, 235-238°.** Recrystd from heptane or toluene. It is a good scintillant. [Hayes et al. *J Am Chem Soc* **77** 1850 *1955*.]

4,4-Bis(4-hydroxyphenyl)valeric acid [diphenolic acid] *[126-00-1]* **M 286.3, m 168-171°, 171-172°, $pK_{Est(1)}$~ 4.8 (CO_2H), $pK_{Est(2)}$~ 7.55 (OH), $pK_{Est(3)}$~9.0 (OH).** When recrystd from $^*C_6H_6$ the crystals have 0.5 mol of $^*C_6H_6$ (**m** 120-122°) and when recrystd from toluene the crystals have 0.5 mol of toluene. Purified by recrystn from hot H_2O. It is sol in Me_2CO, AcOH, EtOH, propan-2-ol, methyl ethyl ketone. It is also recrystallised from AcOH, heptane-Et_2O or Me_2CO + $^*C_6H_6$. It has λ_{max} 225 and 279nm in EtOH. The *methyl ester* has **m** 87-89° (aqueous MeOH to give the trihydrate). [Bader and Kantowicz *J Am Chem Soc* **76** 4465 *1954*.]

Bis(2-mercaptoethyl)sulfone (BMS) *[145626-87-5]* **M 186.3, m 57-58°, pK_1^{25} 7.9, pK_2^{25} 9.0.** Recrystd from hexane as white fluffy crystals. Large amounts are best recrystd from de-oxygenated H_2O (charcoal). It is a good alternative reducing agent to dithiothreitol. Its IR (film) has ν 2995, 2657, 1306, 1248, 1124 and 729 cm^{-1}. The synthetic intermediate *thioacetate* has **m** 82-83° (white crystals from CCl_4). The *disulfide* was purified by flash chromatography on SiO_2 and elution with 50% EtOAc-hexane and recrystd from hexane, **m** 137-139°. [Lamoureux and Whitesides *J Org Chem* **58** 633 *1993*.]

Bombesin (2-L-glutamin-3-6-L-asparaginealytesin) *[31362-50-2]* **M 619.9.** Purified by gel filtration on a small column of Sephadex G-10 and eluted with 0.01 M AcOH. This procedure removes lower molecular weight contaminants which are retarded on the column. The procedure should be repeated twice and the material should now be homogeneous on electrophoresis, and on chromatography gives a single active spot which is negative to ninhydrin but positive to Cl_2 and iodoplatinate reagents. R_F on paper chromatography (*n*-BuOH-pyridine-AcOH-H_2O (37.5: 25:7.5: 30) is 0.55 for Bombesin and 0.65 for Alytin. [Bernardi *Experientia* **B 27** 872 *1971*; **A 27** 166 *1971*.] The *hydrochloride* has **m** 185°(dec) (from EtOH) $[\alpha]_D^{24}$ -20.6° [c 0.65, Me_2NCHO-$(Me_2N)_3PO$ (8:2)].

Bradykinin [ArgProProGlyPheSerProPheArg] *[5979-11-3]* **M_r 1,240.4.** Purified by ion-exchange chromatography on CMC (*O*-carboxymethyl cellulose) and partition chromatography on Sephadex G-25. Purity was checked by paper chromatography using BuOH:AcOH:H_2O (4:1:5) as eluent. [Park et al. *Can J Biochem* **56** 92 1978; ORD and CD: Bodanszky et al. *Experientia* **26** 948 *1970*; activity: Regoli and Barabé *Pharmacol Rev* **32** 1 *1980*.]

Brefeldin A [1-*R*-2*c*,15*c*-dihydroxy-7*t*-methyl-(1*r*,13*t*)-6-oxa-bicyclo[11.3.0]hexadeca-3*t*,11*t*-dien-5-one, Decumbin] *[20350-15-6]* **M 280.4, m 200-202°, 204°, 204-205°, $[\alpha]_D^{22}$ +95° (c 0.81, MeOH).** Isolated from *Penicillium brefeldianum* and recrystd from aqueous MeOH-EtOAc or MeOH. Solubility in H_2O is 0.6mg/mL, 10mg/mL in MeOH and 24.9mg/mL in EtOH. The *O-acetate* recrystallises from Et_2O-pentane and has **m** 130-131°, $[\alpha]_D^{22}$ +17° (c 0.95, MeOH). [Sigg *Helv Chim Acta* **47** 1401 *1964*; UV and IR: Härri et al. *Helv Chim Acta* **46** 1235 *1963;* total synthesis: Kitahara et al. *Tetrahedron* 3021 *1979*; X-ray : Weber et al. *Helv Chim Acta* **54** 2763 *1971*.]

Bromelain (anti-inflammatory Ananase from pineapple) *[37189-34-7]* **M_r ~33 000, [EC 3.4.33.4].** This protease has been purified *via* the acetone powder, G-75 Sephadex gel filtration and Bio-Rex 70 ion-exchange chromatography and has $A_{1cm}^{1\%}$ 20.1 at 280nm. The protease from pineapple hydrolyses benzoyl glycine ethyl ester with a Km (app) of 210mM and k_{cat} of 0.36 sec^{-1}. [Murachi *Methods Enzymol* **19** 273 *1970*; Balls et al. *Ind Eng Chem* **33** 950 *1941*.]

5-Bromo-2'-deoxyuridine *[59-14-3]* **M 307.1, m 193-197°(dec), 217-218°, $[\alpha]_D^{25}$ -41° (c 0.1, H_2O), pK^{25} ~ 8.1.** Recrystd from EtOH or 96% EtOH. It has λ_{max} 279 nm at pH 7.0, and 279 nm (log ε 3.95) at pH 1.9. Its R_F values are 0.49, 0.46 and 0.53 in *n*-BuOH-AcOH-H_2O (4:1:1), *n*-BuOH-EtOH-H_2O (40:11:19) and *i*-PrOH-25% aq NH_3-H_2O (7:1:1) respectively. [*Nature* **209** 230 *1966*; *Collect Czech Chem Comm* **29** 2956 *1964*.]

6-Bromo-2-naphthyl-α-D-galactopyranoside *[25997-59-5]* **M 385.2, m 178-180°, 224-226°, 225°, $[\alpha]_D^{28}$ +60° (c 1.2, pyridine).** It was prepared from penta-*O*-acetyl-D-galactoside and 6-bromo-2-naphthol and $ZnCl_2$. The resulting tetra-acetate (2g) was hydrolysed by dissolving in 0.3N KOH (100mL) and heated until the soln was clear, filtered and cooled to give colourless crystals of the α-isomer which are collected and recrystd twice from hot MeOH. The high specific rotation is characteristic of the α-isomer. The *tetra-acetate* has **m** 155-156° $[\alpha]_D^{20}$ +60° (c 1, $CHCl_3$) [Dey and Pridham *Biochem J* **115** 47 *1969*] [reported **m** 75-85°, $[\alpha]_D^{24}$ +94° (c 1.3, dioxane), Monis et al. *J Histochem Cytochem* **11** 653 *1963*].

5-Bromouridine *[957-75-5]* **M 323.1, m 215-217°, 217-218°, $[\alpha]_D^{25}$ -4.1° (c 0.1, H_2O), pK^{25} 8.1.** Recrystd from 96% EtOH. UV λ_{max} 279nm (log ε 3.95) in H_2O pH 1.9. R_F in *n*-BuOH:AcOH:H_2O (4:4:1) is 0.49; in *n*-BuOH:EtOH:H_2O (40:11:9) is 0.46 and in isoPrOH:25%NH_3:H_2O (7:1:2) is 0.53 using Whatman No 1 paper. [Prystas and Sorm *Collect Czech Chem Commmun* **29** 2956 *1964*.]

Brucine *[357-57-3 (anhydr), 5892-11-5 ($4H_2O$)]* **M 430.5, m 178-179°, $[\alpha]_{546}^{20}$ -149.9° (anhydrous; c 1, in $CHCl_3$), pK_1^{15} 2.50, pK_2^{15} 8.16 (pK_2^{25} 8.28).** Crystd once from water or aq Me_2CO, as tetrahydrate, then suspended in $CHCl_3$ and shaken with anhydrous Na_2SO_4 (to dehydrate the brucine, which then dissolves). Ppted by pouring the soln into a large bulk of dry pet ether (b 40-60°), filtered and heated to 120° in a high vacuum [Turner *J Chem Soc* 842 *1951*]. **VERY POISONOUS**

α-Brucine sulfate (hydrate) *[4845-99-2]* **M 887.0, m 180°(dec).** Crystd from water.

Butyryl choline iodide [(2-butyryloxyethyl)trimethyl ammonium iodide] *[2494-56-6]* **M 301.7, m 85-89°, 87°, 93-94°.** Recrystd from isoPrOH or Et_2O. [Tammelin *Acta Chem Scand* **10** 145 *1956*.] The *perchlorate* has **m** 72° (from isoPrOH). [Aldridge *Biochem J* **53** 62 *1953*.]

S-Butyryl thiocholine iodide [(2-butyrylmercaptoethyl)trimethyl ammonium iodide] *[1866-16-6]* **M 317.2, m 173°, 173-176°.** Recrystd from propan-1-ol and dried *in vacuo*; store in the dark under N_2. The *bromide* has **m** 150° (from Me_2CO) or **m** 140-143° (from butan-1-ol). [Gillis *Chem and Ind (London)* 111 *1957*; Hansen *Acta Chem Scand* **11** 537 *1957*.]

Next Page