THREE NEW ZWITTERIONIC BUFFERING AGENTS*

By M. A. JERMYN†

The two double-zwitterionic buffering agents, PIPPS‡ (1,4-bis(3-sulphopropyl)piperazine) and PIPBS (1,4-bis(4-sulphobutyl)piperazine), have been synthesized. At 18° and 0·05M, PIPPS has apparent pK_{a1} 4·05 and pK_{a2} 8·1 and PIPBS has apparent pK_{a1} 4·6 and pK_{a2} 8·6. The values for pK_{a2} may be compared with that of 6·8 determined elsewhere[1] for PIPES (1,4-bis(2-sulphoethyl)piperazine) at 20°. It is apparent that decreasing charge separation facilitates the removal of the second proton.

At 18° the solubility of PIPPS in water is 2·52 g/100 ml, and of PIPBS, 1·36 g/100 ml. In contrast to the exceedingly insoluble[1] PIPES, it is therefore possible to make use of buffers involving the pK_{a1} range of PIPPS and PIPBS at physiological temperatures. The solubility in water of both PIPBS and PIPPS is much lower at cold-room temperatures, being 0·3 g/100 ml at 1° for PIPBS. Hence PIPBS crystallizes out from PIPBS/KOH buffer of pH 4 in the cold, although this process is much delayed in the absence of seeding.

Reaction between ethylenediamine and propanesultone leads to a product analysing as the hemihydrate of an ethylenediaminodisulphonic acid. This compound is presumably EDPS (*NN'*-bis(3-sulphopropyl)ethylenediamine) since formation of the *NN* derivative seems unlikely on mechanistic grounds. EDPS had apparent pK_{a1} 6·65 and pK_{a2} 9·8 and a solubility in water of 0·65 g/100 ml at 18°. Judged by its ability to prevent the precipitation of copper from alkaline solutions of pH 10 and above, it is an effective complexing agent.

Experimental

All microanalyses were carried out by the Australian Microanalytical Service, Melbourne. Melting points are uncorrected.

(*a*) *1,4-Bis(3-sulphopropyl)piperazine*

A mixture of piperazine hexahydrate (19·4 g, 0·1 mole), 1,3-propanesultone (30·5 g, 0·25 mole), and water (50 ml) was refluxed (5 min) and then chilled. The product (14·9 g, 45%) was obtained as heavy white *needles* after repeated recrystallization from water, dec. above 400° (Found: C, 36·4; H, 6·7; N, 8·5; S, 19·6; neutral equivalent, 166. $C_{10}H_{22}N_2O_6S_2$ requires C, 36·4; H, 6·7; N, 8·5; S, 19·4%; neutral equivalent, 165·2).

* Manuscript received September 6, 1966.

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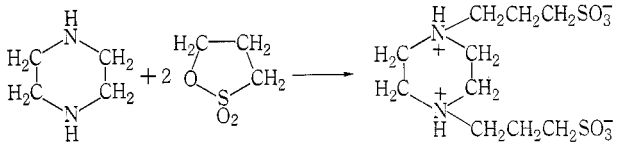
‡ The nomenclature of the trivial names is that of ref. 1.

[1] Good, N. E., Winget, G. D., Winter, W., Connolly, T. N., Izawa, S., and Singh, R. M. M., *Biochemistry*, 1966, **5**, 467.

The overall course of the reaction is



with 1,4-butanesultone replacing the 1,3-propanesultone in the synthesis of PIPBS.

Rather better yields (up to 85%) were obtained if triethylamine (0·2 mole) was added initially to the reaction mixture, which was finally boiled to remove excess triethylamine, chilled, and then adjusted to pH 1·5.

(*b*) *1,4-Bis(4-sulphobutyl)piperazine*

This product was prepared from piperazine hexahydrate (19·4 g, 0·1 mole), 1,4-butanesultone (34·0 g, 0·25 mole), and water (50 ml) by an analogous procedure to the above using 30 min reflux time. It crystallized from water as heavy white *needles* (22·5 g, 60% in the absence of triethylamine; 30·4 g, 85% in its presence), m.p. 370° (dec.) (Found: C, 39·8; H, 7·3; N, 7·7; S, 18·2; neutral equivalent, 178. $C_{12}H_{26}N_2O_6S_2$ requires C, 40·2; H, 7·3; N, 7·8; S, 17·9%; neutral equivalent, 179·2).

(*c*) *NN′-Bis(3-sulphopropyl)ethylenediamine Hemihydrate*

This product was prepared from ethylenediamine (6·0 g, 0·1 mole), 1,3-propanesultone (30·5 g, 0·25 mole), and water (50 ml) by a procedure analogous to (*a*). The initial crystallization from the reaction mixture was very slow. The product (22·2 g, 71%) was obtained as heavy white *needles* after recrystallization from water, m.p. 280° (dec.). It was dried *in vacuo* (4 hr at 60° over P_2O_5) before analysis (Found: C, 30·4; H, 6·9; N, 8·8; S, 20·6; neutral equivalent, 156. $C_8H_{20}N_2O_6S_2,\frac{1}{2}H_2O$ requires C, 30·7; H, 6·8; N, 8·9; S, 20·5%: neutral equivalent, 156·7).