The Nature of Glutaraldehyde in Aqueous Solution

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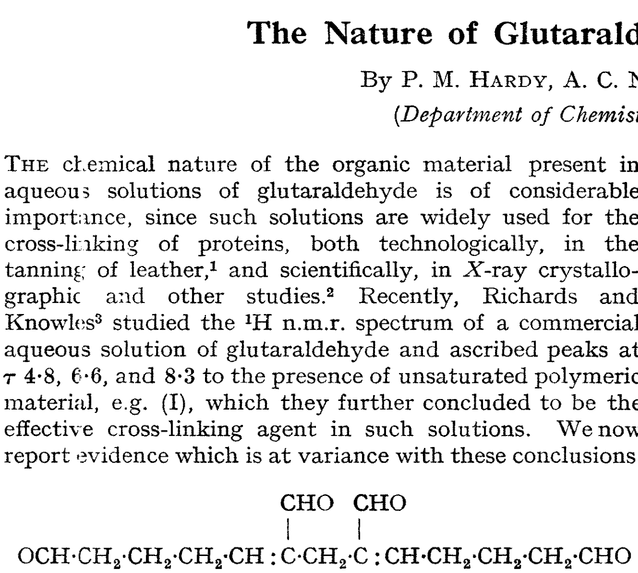
THE chemical nature of the organic material present in aqueous solutions of glutaraldehyde is of considerable importance, since such solutions are widely used for the cross-linking of proteins, both technologically, in the tanning of leather,[1] and scientifically, in *X*-ray crystallographic and other studies.[2] Recently, Richards and Knowles[3] studied the ^{1}H n.m.r. spectrum of a commercial aqueous solution of glutaraldehyde and ascribed peaks at τ 4·8, 6·6, and 8·3 to the presence of unsaturated polymeric material, e.g. (I), which they further concluded to be the effective cross-linking agent in such solutions. We now report evidence which is at variance with these conclusions.



$$\mathrm{OCH{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH{:}\overset{\overset{CHO}{|}}{C}{\cdot}CH_2{\cdot}\overset{\overset{CHO}{|}}{C}{:}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CHO}$$

(I)

The ^{1}H n.m.r. spectra (60 MHz.; 33·5°) of two commercial 25% aqueous solutions of glutaraldehyde showed peaks in approximately the same positions, *viz.* τ 0·30 (triplet), 4·72 (complex), 6·6 (complex), 7·41 (broad triplet) and 8·35 (broad multiplet), as those observed by Richards and Knowles,[3] but with relative areas which differed in the two samples (1 : 1·8 : 1·1 : 1·7 : 14·9 and 1 : 3·6 : 0·8 : 2·4 : 17·3) and from those found by Richards and Knowles (1 : 2 : 3 : 3 : 18). The presence of unsaturated material, probably $\alpha\beta$-unsaturated aldehyde, in our two commercial solutions was confirmed by a relatively weak light absorption at 235 nm.; the intensity of this absorption ($E^{1\%}_{1\,\mathrm{cm}} = 1{\cdot}5$ and 6·2) shows, however, that this can only be a very minor component of the organic content of the solutions. Furthermore, saturation of the solution with sodium chloride, extraction with ether, and distillation eliminates this unsaturated material and gives a 50% yield of monomeric glutaraldehyde with the expected ^{1}H n.m.r. spectrum (triplet at τ 0·29, $\mathrm{C}H\mathrm{O}$; triplet of doublets at τ 7·46, $\mathrm{C}H_2{\cdot}\mathrm{CHO}$; multiplet at τ 8·1, $\mathrm{CH_2{\cdot}C}H_2\mathrm{{\cdot}CH_2}$; relative areas 1 : 2 : 1).

On dissolution in water, pure glutaraldehyde undergoes very rapid hydration. The ^{1}H n.m.r. spectra of solutions in D_2O show peaks at τ 0·28 (triplet), 4·70 (broad triplet), 5·0 (complex), 7·40 (broad triplet) and 8·3 (broad complex); there is no peak at τ 6·6. The relative areas of these vary with concentration, as shown in the Table, and are very different from those observed with the, clearly impure, commercial solutions. These results are most satisfactorily interpreted on the basis of equilibria between glutaraldehyde (II) and the three hydrates (III), (IV), and (V); the peak assignments indicated in the formulae are based on those found for glutaraldehyde itself and for the methyl acetals corresponding to (III) and (V), prepared by the action of methanolic hydrogen chloride on glutaraldehyde, and on the observations of Greenzaid, Luz, and Samuel[4] on other aldehydes.

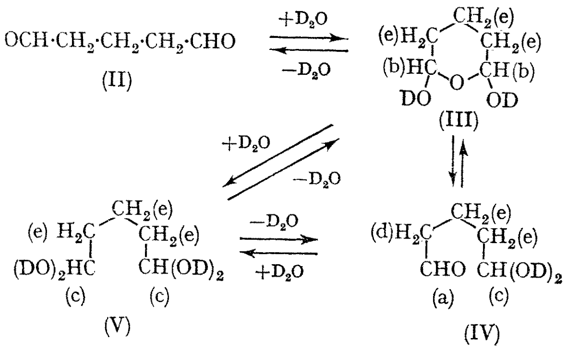
The Table also gives the compositions of the various solutions and the mole-fraction equilibrium constants, K_X, for the equilibria (III) $\rightleftharpoons$ (IV) and (III) $\rightleftharpoons$ (V); that for

TABLE

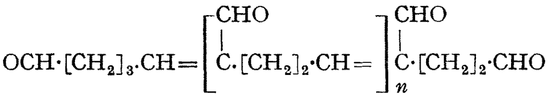
^{1}H *n.m.r. spectra and composition of solutions of glutaraldehyde in* D_2O

Glutaraldehyde (% v/v.)	Glutaraldehyde (mol. l^{-1})	Peak areas (protons) a (τ 0·28)	b (τ 4·70)	c (τ 5·0)	d (τ 7·40)	e (τ 8·3)	Composition (mol. l^{-1}) (III)	(IV)	(V)	K_x (III)⇌(IV)	(III)⇌(V)
2	0·213	0·36	0·65	1·02	0·91	5·08	0·0690	0·0895	0·0575	1·30	0·84
20	2·13	0·38	0·55	1·06	0·80	5·22	0·586	0·852	0·703	1·45	1·26
40	4·25	0·38	0·65	0·96	0·73	5·27	1·38	1·57	1·27	1·14	1·03
60	6·38	0·35	0·70	0·91	0·79	5·25	2·23	2·42	1·72	1·09	0·99
									Mean	1·25	1·03

(II) ⇌ (III) is much higher, there being very little free glutaraldehyde in the solutions. Other possible equilibria which we have considered do not yield satisfactory equilibrium constants over the range of concentrations studied. The almost complete hydration of glutaraldehyde in aqueous solution suggested by these results is very different from the much lesser degree of hydration observed for monofunctional aliphatic aldehydes;[4,5] this is no doubt due to the ease with which the two ends of the glutaraldehyde molecule can approach one another to form the cyclic monohydrate (III). It is only to be expected that this will be in tautomeric equilibrium with the open-chain monohydrate (IV) and that both will be capable of further hydration to the dihydrate (V).



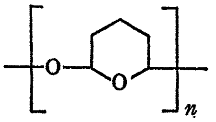
Even after several weeks at room temperature the ^{1}H n.m.r. spectra of the aqueous solutions of glutaraldehyde prepared from the redistilled compound show little change, there still being no peak at τ 6·6 and no appreciable light absorption at 235 nm.; it is evident that these spectroscopic properties of the commercial solutions are due to impurities. The molecular weight of the solute (vapour pressure osmometer) in such solutions was also unchanged over a period of several weeks and it is clear that the polymerisation of glutaraldehyde in neutral aqueous solution is very slow. Cross-linking using such solutions must therefore involve glutaraldehyde itself or one of its hydrates and not an unsaturated polymer as suggested by Richards and Knowles.[3] However, in weakly alkaline solution (pH 8) glutaraldehyde rapidly polymerises to an insoluble solid, the spectroscopic properties of which, especially the light absorption at 235 nm. ($E^{1\%}_{1\,\mathrm{cm.}}$ = 31·5), are consistent with unsaturated structures such as (I) or (VI) or intermediates between these. It is possible, therefore, that cross-linking reactions in alkaline solution may involve such unsaturated polymers.



$$OCH\cdot[CH_2]_3\cdot CH{=}\left[\overset{CHO}{\overset{|}{C}}\cdot[CH_2]_2\cdot CH{=}\right]_n\overset{CHO}{\overset{|}{C}}\cdot[CH_2]_2\cdot CHO$$

(VI)

Distilled glutaraldehyde, alone or dissolved in chloroform or benzene, undergoes rapid polymerisation, apparently catalysed by traces of water, to a glassy solid. The spectroscopic properties (^{1}H n.m.r.; i.r.) of this are in accordance with the acetal-like structure (VII), originally suggested by Aso and Aito.[6] This polymer, unlike that formed in alkaline solution, reverts to monomer on heating and the aldehyde peak in its ^{1}H n.m.r. spectrum at τ 0·21, which is reduced in area by reprecipitation from dioxan with water may be due to the presence of free glutaraldehyde rather than the pendant aldehydo-groups suggested by these authors. The polymers formed by glutaraldehyde in aqueous acid may be largely of this type.



(VII)

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