C=C 6.17 μ (m); uv $\lambda_{max}$ (EtOH) 253 nm (ε 5810), 284 (1210); pmr δ 6.88 (dd, 1, *J* = 2, 7 Hz, H-12), 7.02 (dt, 1, *J* = 2, 7 Hz, H-10), 7.15 (dt, 1, *J* = 2, 7 Hz, H-11).

*Anal.* Calcd for $C_{17}H_{20}O_2N_2$: C, 71.82; H, 7.09; N, 9.85. Found: C, 71.61; H, 7.19; N, 10.03.

A solution of 110 mg of **10dA** in 10 ml of 10% aqueous acetic acid was refluxed for 20 hr and then cooled, neutralized with solid potassium bicarbonate, and extracted with methylene chloride. The extract was dried and evaporated. The pmr spectrum of the oily residue, 102 mg, showed the presence of a 1.6:1 mixture of **10dA** and **10dB**, respectively. A solution of 100 mg of **10dB** in 10 ml of 10% aqueous acetic acid was refluxed for 20 hr. Similar reaction work-up led to 77 mg of product whose tlc (silica gel, 1.6:1 chloroform-methanol) revealed **10dA** and **10dB**, the former predominating, and whose pmr spectrum exhibited signals of a 1.9:1 mixture of **10dA** and **10dB**, respectively.

**Registry No.**—**3b**, 4695-82-3; **4c**, 2671-38-7; **5**, 51240-38-1; **6b**, 51240-39-2; **8**, 13861-75-1; **9a**, 51240-40-5; **9b**, 51240-41-6; **10aA**, 30671-33-1; **10bA**, 51268-47-4; **10cA**, 51240-42-7; **10dA**, 51240-43-8; **10bB**, 51268-48-5; **10dB**, 51268-49-6; tryptophol, 526-55-6; ketochloroindolenine, 51240-44-9.

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(20) The indolizidine portion of **10aA** and **10aB** is portrayed usually with C(5), C(6), C(7), and C(3) in coplanar relationship.[13,19] Since this conformation exposes the geminal C(7) substituents to an energetically unfavorable, eclipsing interaction with the C(6) hydrogens, coplanarity between $N_b$, C(5), C(6), and C(7) is preferred. This latter representation has been illustrated previously without explanation.[15]
(21) All alkaloids have been assumed to possess a *trans*-indolizidine structure in all solutions on the basis of a cis structure being destabilized by extra 1,3-diaxial interactions in the piperidine moiety.[19] While infrared and pmr spectral analyses of simply substituted indolizidines indicate the basic skeleton to be trans fused [H. S. Aaron and C. P. Ferguson, *Tetrahedron Lett.*, 6191 (1968); T. A. Crabb, R. F. Newton, and D. Jackson, *Chem. Rev.*, **71**, 109 (1971)], the difference of energy between the cis and trans forms is too low and the steric as well as polar interactions of the oxindole ring with the indolizidine nucleus too subtle and complex to permit acceptance of a trans configuration for the latter in compounds **7** without rigorous proof. Equilibration in pyridine leading to **10aA** has been assumed to be the consequence of electron repulsion between the carbonyl oxygen and $N_b$, while preference of **10aB** in acetic acid has been ascribed to hydrogen bridging of a $N_b$-protonated species with the carbonyl oxygen.[13,19] However this explanation appears to be oversimplified in view of the fact that the isomer ratio rarely ever exceeds 3:1, indicating a small energy difference, and that substances **7** can be expected to be only minimally protonated in aqueous acetic acid. Merely differences in solvation suffice to give variations of equilibrium positions.
(22) The same configurations were assigned earlier[15] on the basis of pmr data on **7a** with assumed *trans*-indolizidine structures.
(23) For convenience the oxindole alkaloid nomenclature is being maintained for the models **7**.
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# Structure of Aqueous Glutaraldehyde

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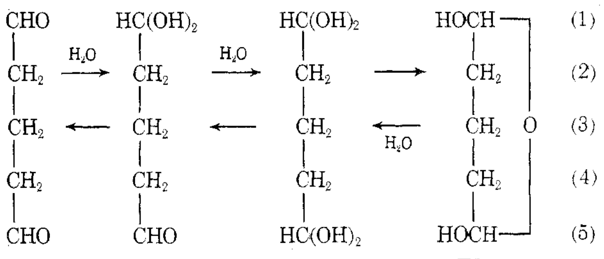
The carbon-13 nmr spectrum of aqueous 25% glutaraldehyde has been assigned to individual components in an equilibrium mixture. The solution is shown to consist primarily of the cyclic hemiacetal, equally present in its two isomeric forms, in equilibrium with the free aldehyde. The ratio of these components varies strongly with temperature. Approximately 25% of the mixture is present as the linear hemihydrate and the dihydrate in about a 2:1 ratio, this fraction being much less temperature dependent. Higher order oligomers contribute very little to the equilibrium mixture.

The structure of gluataraldehyde (pentanedial) in aqueous solution, in which form it is available as a commercial product,[1] has been the subject of several proton magnetic resonance studies,[2-4] not all of which give consistent results. The currently accepted structure,[4] based on the work by Hardy, Nicholls, and Rydon,[3] is that the solution contains roughly equal amounts of the hemihydrate II, the dihydrate III, and the cyclic hemiacetal IV.



I II III IV

It has not been possible to distinguish individual structures in the proton magnetic resonance spectrum owing to the overlapping of complex bands. Carbon-13 nmr is much less subject to these restrictions. With broad band proton decoupling, I, III, and IV should give simple three-line, 2:2:1 intensity patterns, and five equally intense lines should result from II. Higher order oligomers or condensa-

**Table I**
**Carbon-13 Line Positions and Assignments in Aqeous Glutaraldehyde**

| C3 band | | | C2 and C4 band | | | C1 and C5 band | | |
|---|---|---|---|---|---|---|---|---|
| Line[a] | Shift[b] | Assignment[c] | Line | Shift | Assignment | Line | Shift | Assignment |
| 1 | 14.6 | I | 7 | 29.4 | ? | 15 | 91.35 | III |
| 2 | 16.9 | IVb | 8 | 30.9 | IVb | 16 | 91.6 | II |
| 3 | 17.1 | II | 9 | 31.65 | IVa | 17 | 92.3 | IVb |
| 4 | 17.3 | III | 10 | | ? | 18 | 95.3 | IVa |
| 5 | 19.4 | IVa | 11 | 36.7 | II (2) | 19 | 208.4 | I |
| 6 | 20.25 | ? | 12 | 37.1 | III | 20 | 209.1 | II (5) |
| | | | 13 | 42.5 | I | | | |
| | | | 14 | 43.0 | II (4) | | | |

[a] Figure 1. [b] Parts per million downfield from external TMS. Internal dioxane occurs at 66.4 ppm on this scale. [c] See text.

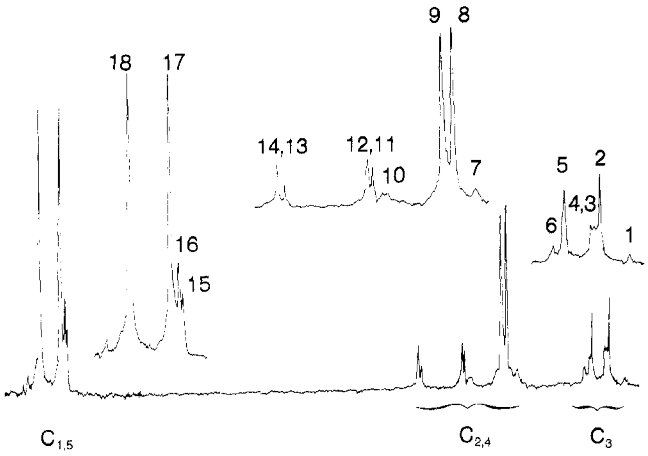
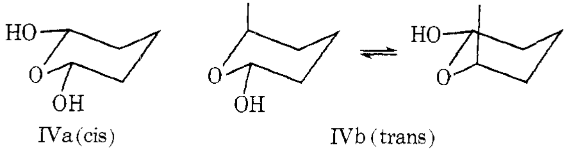


**Figure 1.** Carbon-13 nmr spectrum (exclusing aldehyde lines) of aqueous 25% glutaraldehyde at 23° (22.63 MHz, increasing frequency from right to left). Line assignments are given in Table I.

tion products should give more complex patterns than any of these, and therefore be fairly easy to recognize. There is also no possibility of solvent interference.

The 22.63-MHz, proton-decoupled, naturally abundant carbon-13 spectrum of an aqueous, 25% solution of repurified (saturate commercial solution with NaCl, extract with ether, dry, strip, and distil[3]) glutaraldehyde is shown in Figure 1, and the line assignments are detailed in Table I.[5] The commercial material shows substantially the same spectrum. The assignments are based on chemical shift additivity rules[6] and changes in the line intensities that occur as the result of variation in concentration and temperature.

The two major components in solution each give rise to a 2:2:1 pattern whose chemical shifts all fall far outside the range of free aldehyde groups. While this would be generally consistent with Hardy, Nicholls, and Rydon's conclusion that III and IV are present in large quantity, we believe that these patterns instead represent the two geometrical isomers of IV, *i.e.*, IVa and IVb.



IVa(cis) IVb(trans)

This is based on three main lines of argument. The first is that conformational energy considerations (anomeric effects) would lead one to expect both isomers to be present in comparable amounts of aqueous solution, while the driving force for the formation of III is much less apparent. The second is that the relative chemical shifts between the two observed species, particularly the ~2.5 ppm difference at carbons 1 and 3 *vs.* the smaller difference of 0.7 ppm at $C_2$, are in accord with the well-known $\gamma$ effect in cyclic systems.[7] Thirdly, the relative intensities of the two principal patterns do not change significantly between 25 and 50% aqueous glutaraldehyde, although the molarity of water decreases by approximately 10%. The equilibrium between III and IV, which involves an additional molecule of water, should be affected, while that between IVa and IVb would not.

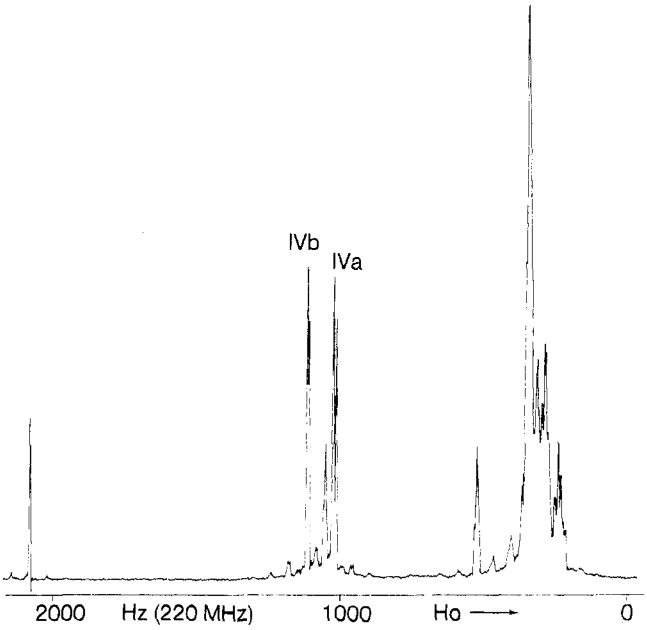


**Figure 2.** 220-MHz proton magnetic resonance spectrum of purified glutaraldehyde (25% in $D_2O$).

The assignment of the principal low-temperature structures to the two isomers of the cyclic hemiacetal, IVa, and IVb, is confirmed by the 220-MHz proton spectrum, Figure 2. Two strong, equally intense bands occur in the central region characteristic of $-CH(OR)_2$ groups, both of which show a principal doublet structure ($J$ = 6, $J'$ = 2 Hz and $J$ = 9.2, $J'$ = 2 Hz). The dihydrate would have to show triplet fine structure since the two adjacent methylene protons are isochronous and strongly coupled. IVa should exist in the diequatorial form, so that the anomeric proton would experience one strong ($J_{aa} \cong$ 10 Hz) and one weak ($J_{ae} \cong$ 2-4 Hz) vicinal coupling. Since axial protons generally occur at higher field than equatorial, this would correspond to the upper band, which has the larger doublet splitting. Isomer IVb, which gives the lower field band, is the weighted average of two interconverting conformations, and should have vicinal coupling constants equal to $\frac{1}{2}(J_{aa} + J_{ee})$ and $\frac{1}{2}(J_{ae} + J_{ea})$, respectively. The larger splitting is accordingly reduced by 30-40%, as is observed, and the smaller coupling is not substantially affected.

**Table II**
**Predicted[a] Chemical Shifts of Glutaraldehyde Hydrates**

| Compd | Carbon number 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| *n*-Pentane[b] | 13.7 | 22.6 | 34.5 | 22.6 | 13.7 |
| I | 202.0[c] | 51.6 | 34.5 | 51.6 | 202.0[c] |
| II | 110.3 | 41.0 | 22.9 | 53.6 | 202.0[c] |
| III | 110.3 | 43.0 | 11.3 | 43.0 | 110.3 |
| Pyran[d] | 69.7 | 27.9 | 25.1 | 27.9 | 69.7 |
| IVa | 111.8 | 34.2 | 22.9 | 34.2 | 111.8 |
| IVb | 105.4 | 33.5 | 17.2 | 33.5 | 105.4 |

[a] Based on reference compounds listed and additive corrections (ref 6)

|  | $\alpha$ | $\beta$ | $\gamma$ |  | $\alpha$ | $\beta$ | $\gamma$ | $\delta$ |
|---|---|---|---|---|---|---|---|---|
| –OH | 48.3 | 10.2 | −5.8 | –OH(e) | 43.2 | 7.9 | −1.1 | −1.6 |
| –CHO |  | 31 | −2 | –OH(a) | 37.8 | 5.5 | −6.8 | −0.7 |

for linear and cyclic derivatives, respectively. [b] D. M. Grant and E. G. Paul, *J. Amer. Chem. Soc.*, **86,** 2984 (1964). [c] *n*-Hexyl aldehyde value, converted to TMS reference: J. B. Stothers and P. C. Lauterbur, *Can. J. Chem.*, **42,** 1563 (1964). [d] G. E. Maciel and G. B. Savitsky, *J. Phys. Chem.*, **69,** 3925 (1965).

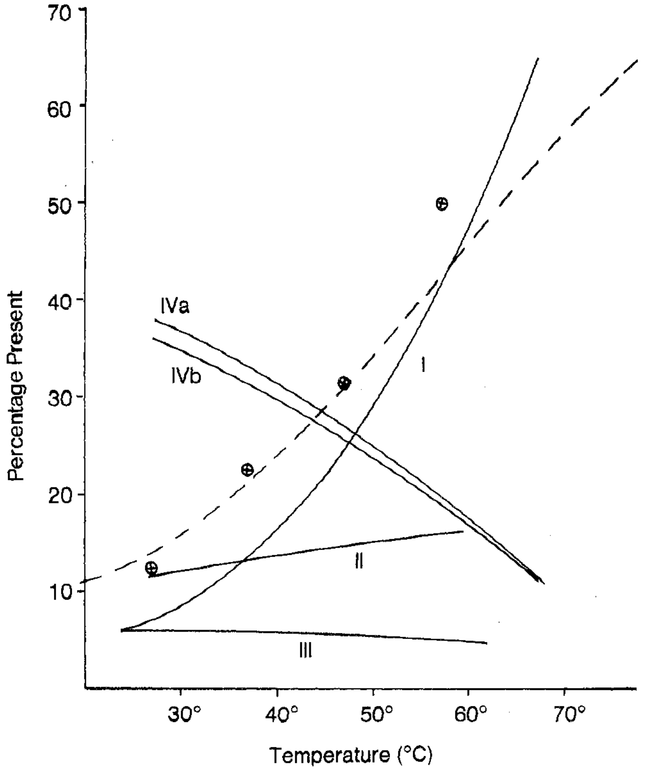


**Figure 3.** Composition (solid curves, $^{13}$C nmr) and percentage of free aldehyde groups (broken curve, pmr[4]) in aqueous 25% glutaraldehyde *vs.* temperature. Free aldehyde fractions calculated from the composition are indicated by points x.

These spin coupling effects are not so clear in the proton spectra in lower magnetic fields, since they require both that the anomeric protons be clearly resolved and that the chemical shifts between the adjacent methylene protons be large with respect to the geminal coupling constant between them in order to eliminate virtual coupling effects.

A secondary set of five equally intense carbon-13 lines occurs that can be assigned to the hemihydrate II. It is considerably less abundant than previously asserted,[3] and each of its lines is accompanied by a weaker, secondary component. We assign these weaker lines to a mixture of the dihydrate III and the free aldehyde I. The chemical shifts, while not accurately predicted by additive substituent rules (Table II), do show consistent internal trends. The lines due to the free aldehyde could be positively identified, since its concentration has been shown to increase with elevation of the temperature.[4] Three weak, somewhat broader bands remain unassigned, and are probably due to oligomers.

Based on the peak heights of lines 8, 9, 11, 12, and 13 of the carbon-13 spectrum, we estimate the following composition for purified aqueous glutaraldehyde in neutral solution at 23°: I, 4%; II, 16%, III, 9%; IVa, 35%; IVb, 36%. This varies slightly with concentration and pH, and strongly with temperature. The approximate composition of commercial 25% aqueous glutaraldehyde[1] is plotted as a function of temperature in Figure 3. Also shown is the fraction of free aldehyde groups calculated from previously available pmr data,[4] and the corresponding points (x) that are determined from the composition as estimated by carbon-13; *i.e.*, ½ II + I. The agreement between the independent measurements and samples, particularly at low temperatures, is reasonably good.

In summary, we conclude that aqueous glutaraldehyde consists primarily of the cyclic hemihydrate, equally distributed between its two stereoisomeric forms; that the proportion of the linear dihydrate is about 25% as high, and the hemihydrate about 50% as high as previously proposed;[3] and that, at the temperatures originally cited (~40°), the contribution of free aldehyde to the equilibrium mixture has been substantially underestimated.

**Registry No.**—I, 110-30-8; II, 51052-01-8; III, 51052-02-9; IVa, 51052-03-0; IVb, 51052-04-1.

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