960. *The Structure of Two Trimers of Biacetyl.*

By R. M. CRESSWELL, W. R. D. SMITH, and H. C. S. WOOD.

The preparation of a new trimer of biacetyl is described and, on the basis of its physical and chemical properties, structure (I) is suggested for this compound. The chemistry of a second trimer of biacetyl has been re-examined and a new structure (VII), based largely on physical evidence, is proposed.

WE recently described[1] the condensation of a new trimer of biacetyl with 5-amino-4-D-ribitylaminouracil to give riboflavin and 6,7-dimethyl-8-D-ribityl-lumazine. We suggested that this reaction might be related to the biosynthesis of riboflavin and of the pteridine derivative. We now describe the preparation of this trimeric biacetyl and experiments which lead us to suggest structure (I) for this compound.

Biacetyl, when kept for 4—5 days at room temperature in contact with an anion-exchange resin (Amberlite CG-400, OH form), undergoes self-condensation; the crystalline product is the same as separates from some specimens of biacetyl which have been stored for long periods. Elementary analysis and molecular-weight determination indicate that the compound is a trimer, $C_{12}H_{18}O_6$. It differs, however, from that previously described.[2]

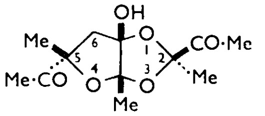
The infrared spectrum shows strong bands (in CCl_4) at 1724 (C=O) and 3460 cm.$^{-1}$ (OH). The nuclear magnetic resonance spectrum shows bands at 5·52 (hydroxyl proton), 7·741 and 7·804 (two different $\mathrm{C}H_3\cdot\mathrm{CO}$), 8·608, 8·682, and 8·708 (three $\mathrm{C}H_3\text{–}\mathrm{C}\overset{\diagup}{\underset{\diagdown}{-}}\mathrm{O}\text{–}$), and two doublets at 6·87 and 8·24 τ ($\text{–}\mathrm{C}H_2\text{–}$; AB type, $J_{AB} = 13{\cdot}5$ cycles/sec.). The position of the hydroxyl band in these spectra indicates that there is relatively strong hydrogen bonding in the molecule, and as the position of this band in the infrared spectrum is not affected by dilution we conclude that the bonding is intramolecular. It is to be expected that there would also be two bands in the 6 μ region corresponding to bonded and unbonded

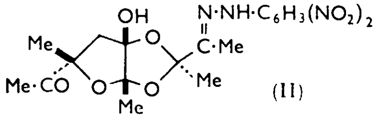
[1] Cresswell and Wood, *J.*, 1960, 4768.
[2] Diels and Jost, *Ber.*, 1902, **35**, 3290.

carbonyl groups; we have, however, been unable, even by using a calcium fluoride prism and slow scanning speeds, to split the single band at 1724 cm.$^{-1}$.

The trimer is very unstable and it has been possible to prepare only one derivative of the intact molecule. Treatment with an excess of 2,4-dinitrophenylhydrazine sulphate in methanol gave, almost immediately, a yellow monodinitrophenylhydrazone whose infrared spectrum (in Nujol) showed a strong band at 1709 cm.$^{-1}$ (C=O). The spectrum in the 2—4 μ region of a dilute solution in carbon tetrachloride (1 cm. cells) showed absorption characteristic of an unbonded hydroxyl group (3610 cm.$^{-1}$). This derivative recrystallised unchanged from nitrobenzene, but in a hydroxylic solvent such as ethanol hydrolysis gave the monodinitrophenylhydrazone of biacetyl as the only isolable product. We conclude that the original dinitrophenylhydrazone is best represented by structure (II), and that hydrolysis proceeds by successive breaking of acetal linkages to give the monodinitrophenylhydrazone of biacetyl and biacetyl dimer (III or the related open-chain hexanetrione). We have confirmed the presence of this dimer in the ethanolic mother liquors from the above recrystallisation by addition of a 4-alkylamino-5-aminouracil, obtaining pteridines analogous to those obtained in our previous work [1] and identified by paper chromatography.

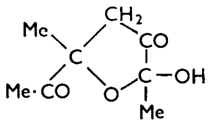
(I)

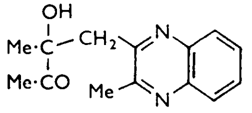




(II)

(III)





(IV)

Attempts to prepare other carbonyl or hydroxyl derivatives resulted in disruption, giving in most cases simple derivatives of biacetyl.

In aqueous solution of pH >8 the trimer behaves like a mixture of biacetyl and the aldol (III) (or the related open-chain hexanetrione). Thus, we have already reported [1] that it condenses with 4,5-diaminouracil, giving 6,7-dimethyl-lumazine and 2,4-dihydroxy-7-(2-hydroxy-2-methyl-3-oxobutyl)-6-methylpteridine. Similarly with *o*-phenylenediamine it gives 2,3-dimethyl- and 2-(2-hydroxy-2-methyl-3-oxobutyl)-3-methyl-quinoxaline (IV), the latter identical with a specimen prepared from the dimeric aldol (III) and *o*-phenylenediamine.

Structure (I) is derived from the dimer (III), the third molecule of biacetyl being attached by successive acetal linkages. There is, however, the possibility that the trimer is derived from an open-chain structure (V): successive ring closures involving acetal linkages could lead to structure (VI) or to two different bicyclo[3,2,1]systems. We have eliminated the last two possibilities because Dreiding models show that the distance between the hydroxyl group and either carbonyl group is too great to permit the hydrogen bonding indicated by the spectra. We have also eliminated structure (VI) which contains an α-hydroxy-ketone grouping since the trimer does not react with aqueous sodium periodate. This estimation was carried out by titration of the periodate in mineral acid solution with sodium thiosulphate and potassium iodide; [3] the standard technique [4] which involves titration with sodium arsenite in aqueous sodium hydrogen carbonate solution was not here feasible as at this pH the trimer dissociates rapidly (see above) into biacetyl

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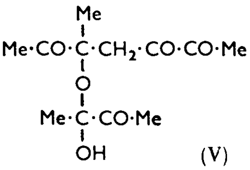
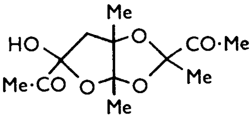
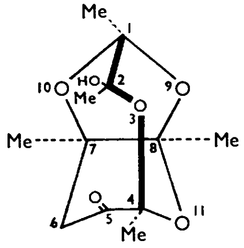
[3] Scott, "Standard Methods of Chemical Analysis," 5th Ed., Vol. I, van Nostrand, New York, 1939, p. 458.

[4] Jackson, *Organic Reactions*, Vol. 2, p. 361.

and the aldol (III), each of which then reacts with one equivalent of periodate giving an apparent " uptake " by the trimer molecule of two mol. of periodate.


(V)


(VI)



(VII)

The stereochemistry at positions 2 and 5 of the bicyclic ring system (I) must also be as shown for the following reason. The infrared spectrum of the monodinitrophenylhydrazone (II) shows no evidence of hydrogen bonding between carbonyl and hydroxyl groups, whereas in the trimer (I) strong hydrogen bonding is present. Therefore the carbonyl group attached at position 2 must be on the same side of the ring as the hydroxyl group, and that attached to position 5 must be on the opposite side. This configuration also explains why only one of the carbonyl groups reacts with dinitrophenylhydrazine, since the 5-acetyl group is sterically hindered by the 2-methyl group.

We have also re-examined the other trimer of biacetyl, first prepared by Diels and Jost [2] by treatment of biacetyl with concentrated hydrochloric acid at 0°. This compound shows infrared bands (CCl_4 solution) at 1727 (C=O) and 3610 cm.$^{-1}$ (unbonded OH). The nuclear magnetic resonance spectrum shows bands at 7·40 (hydroxyl proton), 8·45, 8·47, 8·59, 8·62, and 8·72 (five $CH_3{-}C{\leqslant}O{-}$), and two doublets at 6·83 and 7·54 τ ($-CH_2{\cdot}CO-$ AB type, $J_{AB} = 10{\cdot}0$ cycles/sec.). This evidence is incompatible with Diels and Jost's structure [2] and we suggest that this trimer has structure (VII). This is formed from the open-chain precursor (V) by successive acetal linkages. It is in agreement with Diels and Jost's report of monocarbonyl (oxime, semicarbazone etc.) and alcohol derivatives (monoacetate, phenylurethane). The stereochemistry at the carbon atom which carries the hydroxyl group is also defined, since the hydroxyl group must be remote from the carbonyl group to avoid hydrogen bonding. Structure (VII) is the only one which can accommodate the physical and chemical properties described above if we make the reasonable assumption that, in the formation from the precursor (V) of the *initial* acetal link, ring closure to give a 7-membered ring will not take place where formation of a 5-membered ring [which leads eventually to structure (VII)] or of a 6-membered ring (which leads to products which do not agree with the physical evidence) is possible.

EXPERIMENTAL

Infrared Spectra.—These were determined with a Grubb-Parsons spectrophotometer and sodium chloride and calcium fluoride prisms. The solvent was carbon tetrachloride in all cases except one indicated in the text.

Nuclear Magnetic Resonance Spectra.—The spectrum of compound (I) was determined with a Varian V-4300-2 spectrometer with a 40·0 Mc. oscillator. The spectrum, which was determined for a 6% solution in carbon tetrachloride, was calibrated by the side-band technique. The spectrum of compound (VII) was determined with a Varian A-60 spectrometer for a solution in deuterochloroform. Tetramethylsilane (1%) was added as an internal reference in each case.

Paper Chromatography.—Chromatograms were developed by the technique and solvent systems previously described.[1]

2,5-*Diacetyl*-3a,5,6,6a-*tetrahydro*-6a-*hydroxy*-2,3a,5-*trimethylfuro*[2,3-d]-1,3-*dioxole* (I).—An anion-exchange resin (Amberlite CG-400) was converted into its hydroxyl form by washing it with 5% sodium hydroxide solution. The resin was washed free from alkali with distilled water (carbonate-free), dried at 30°, and used within 24 hr.

The resin (10 g.) was added portionwise to biacetyl (30 c.c.) during 30 min., any rise in temperature being checked by external cooling. The mixture was kept for 5 days at room temperature with periodic mixing, a solid mass being obtained. Ether (100 c.c.) was added, the resin was removed by filtration, and the ether *in vacuo*. Water (3 c.c.) was added to the resulting pale yellow gum, which crystallised overnight at 0°. Recrystallisation from ether gave the ***trimer*** (7·5 g.) as colourless needles, m. p. 73—75° [Found: C, 55·9; H, 7·2%; *M* (ebullioscopic in C_6H_6), 251, 253. $(C_4H_6O_2)_3$ requires C, 55·8; H, 7·0%; *M*, 258]. *X*-Ray crystallography showed the crystal to be monoclinic and indicated that the unit cell contained 12 biacetyl units. This result also supports a trimeric structure.

The trimer (1 g.), dissolved in the minimum amount of methanol, was added to a solution of 2,4-dinitrophenylhydrazine (2·5 g.) and sulphuric acid (4 c.c.) in methanol (35 c.c.) and water (10 c.c.). A yellow precipitate was formed almost immediately, and after 10 min. was collected and recrystallised from nitrobenzene, to give the 2,4-*dinitrophenylhydrazone* (1·5 g.) as yellow needles, m. p. 155—156° (Found: C, 49·7; H, 4·9; N, 13·2. $C_{18}H_{22}N_4O_9$ requires C, 49·3; H, 5·1; N, 12·8%).

Attempted recrystallisation from ethanol gave biacetyl monodinitrophenylhydrazone[5] as orange needles, m. p. and mixed m. p. 174—175° (Found: C, 45·0; H, 3·6; N, 20·6. Calc. for $C_{10}H_{10}O_5N_4$: C, 45·1; H, 3·8; N, 21·0%), whose infrared spectrum was as reported.[5]

To the mother liquors from the ethanol recrystallisation was added a solution of 5-amino-4-2′-hydroxyethylaminouracil.[1] The mixture was heated on the steam bath for 15 min. and examined by paper chromatography. The mixture of pteridines obtained was identical with that formed[1] similarly from dimeric biacetyl (III).

2-(2-*Hydroxy*-2-*methyl*-3-*oxobutyl*)-3-*methylquinoxaline* (IV).—5-Acetyltetrahydro-2-hydroxy-2,5-dimethyl-3-oxofuran (III) (1·7 g.) in water (15 c.c.) was heated with *o*-phenylenediamine (1·08 g.) in water (10 c.c.) on the steam bath for 10 min. The pale yellow precipitate which separated on cooling recrystallised from aqueous ethanol, to give the *quinoxaline* (1·8 g.) as pale yellow needles, m. p. 110—112°. Sublimation at 110° (bath)/0·05 mm. gave colourless needles, m. p. 112—114° (Found: C, 68·6; H, 6·8; N, 11·3. $C_{14}H_{16}O_2N_2$ requires C, 68·8; H, 6·6; N, 11·5%).

Condensation of Trimeric Biacetyl (I) *with* o-*Phenylenediamine*.—Trimeric biacetyl (I) (0·6 g.) was heated with *o*-phenylenediamine (0·5 g.) in water (20 c.c.) on the steam bath for 5 min., then cooled and extracted with ether (3 × 50 c.c.). The extracts were dried (Na_2SO_4) and evaporated, giving a gum (0·85 g.) that was chromatographed in benzene on neutral alumina (Woelm; 25 g.). Benzene (200 c.c.) eluted a fraction (0·29 g.) which crystallised from aqueous ethanol, to give 6,7-dimethylquinoxaline,[6] m. p. and mixed m. p. 100—102°. Ether containing 1% of methanol (150 c.c.) eluted a second main fraction (0·32 g.) which crystallised from aqueous ethanol to give 2-(2-hydroxy-2-methyl-3-oxobutyl)-3-methylquinoxaline, m. p. 110—112°, identical with the material prepared as above.

Trimeric Biacetyl (2-*Hydroxy*-1,2,4,7,8-*pentamethyl*-3,9,10,11-*tetraoxotricyclo*$[5,2,1,1^{4,8}]$-*undecan*-5-*one*) (VII).—This compound was obtained by Diels and Jost's method[2] as colourless needles (from water), m. p. 102—103° (lit., 105°).

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[5] Reich and Hefle, *J. Org. Chem.*, 1956, **21**, 708.

[6] Bost and Towell, *J. Amer. Chem. Soc.*, 1948, **70**, 903.