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A CONTRIBUTION TO THE SKELETAL STRUCTURE OF RYANODINE

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SOME time ago we have reported[1] the hydrolysis of the insecticide ryanodine, $C_{25}H_{35}O_9N$, to pyrrole-α-carboxylic acid and ryanodol, $C_{20}H_{32}O_8$. On treatment with 10% aqueous sulphuric acid overnight, ryanodol eliminates a molecule of water and yields anhydroryanodol[2] which has now been obtained crystalline and recrystallized from acetone-ether (m.p. 244°). Found: C, 62.76; H, 8.06; O, 29.35. Calc. for $C_{20}H_{30}O_7$: C, 62.82; H, 7.91; O, 29.29%. I.R. (KBr): 1723 cm^{-1} (broad).

Anhydroryanodol consumes one mole of periodic acid and yields oxoanhydroryanodol which crystallizes readily from acetone (m.p. 259°). Found: C, 62.92; H, 7.26; O, 29.60. Calc. for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42; O, 29.43%.

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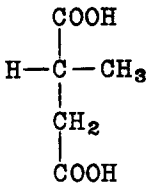
[1] R. B. Kelly, D.J. Whittingham and K. Wiesner, Can. J. Chem. 29, 905 (1951).

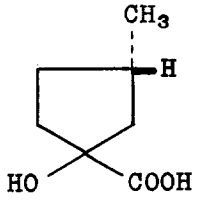
[2] R.B. Kelly, D.J. Whittingham and K. Wiesner, Chem. and Ind., p. 857 (1952).

Ryanodol itself consumes two moles of periodic acid in acidic solution and yields oxoryanodol, m.p. 207°, which may be recrystallized from acetone. Found: C, 58.21; H, 7.14; O, 34.73; C-CH_3, 11.61. Calc. for $C_{20}H_{28}O_8 \cdot H_2O$: C, 58.10; H, 7.30; O, 34.75; 4 C-CH_3, 14.17%. I.R.: 1778 cm^{-1} (γ-lactone), 1718 cm^{-1}. The N.M.R. spectrum showed absence of vinylic hydrogens and of aldehyde groups. A singlet at 1.93 ppm. with an area 1H was assigned to a formate ester group. Such a group must be formed by periodate cleavage of a hemiacetal. Mild alkaline hydrolysis of oxoryanodol gave 0.65 moles of isobutyric acid and formic acid, both identified by partition chromatography on silicic acid. The isobutyric acid was further converted into the crystalline amide, m.p. 128°, which was identical by mixed melting point and infrared spectrum with an authentic specimen.

In phosphate buffer (pH=6.8), ryanodol consumes three moles of periodate in two hours. The product was extracted by ether and chromatographed on silicic acid. The first chloroform fractions contained a compound which crystallized from acetone, m.p. 192°. Found: C, 60.99; H, 6.66; O, 32.10. Calc. for $C_{20}H_{26}O_8$: C, 60.96; H, 6.65; O, 32.49%. I.R. (KBr): 1770 (γ-lactone), 1740, 1696 cm^{-1}. The infrared spectrum in CCl_4 indicated the absence of hydroxy groups. The N.M.R. spectrum showed absence of



(I)



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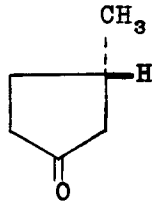

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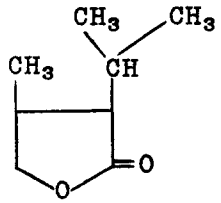
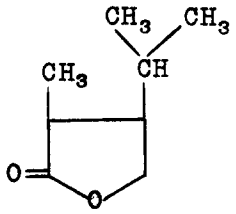
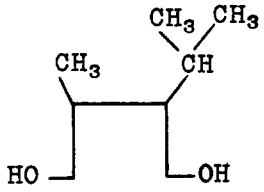


(IVa)



(IVb)



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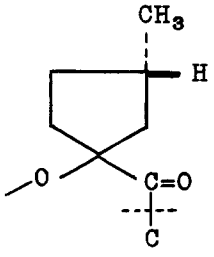
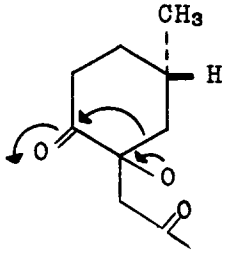
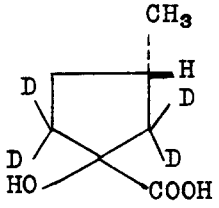


(VI)



(VII)



(VIII)

vinylic hydrogens and aldehydes and a singlet with an area 1H at 1.93 ppm. ascribed to a formate ester. The N.M.R. spectrum further showed five C-methyls with a total area of 15H at 8.60, 8.91, 8.94, 9.03 and 9.19 ppm. This is in agreement with many Kuhn-Roth determinations on various ryanodol derivatives which indicate the presence of three to four C-methyls, and with the known presence of a geminal dimethyl group.

From the results of the periodate cleavages it appears probable that ryanodol has at least six hydroxyls. This would mean that the skeleton contains three carbon rings and two cyclic ethers. We have already reported[2] that an alkaline hydrolysis of anhydrooxoryanodol yields almost a full mole of isobutyric acid. We have now repeated the hydrolysis of oxoanhydroryanodol by 5% ethanolic potassium hydroxide under reflux for 6 hours on a large scale. The acidic products of this reaction were extracted, hydrogenated with platinum oxide in acetic acid and separated by partition chromatography on silicic acid into three fractions eluted by (a) chloroform, (b) 5% butanol in chloroform, and (c) 20% butanol in chloroform.

The fraction (c) was crystalline and after crystallization from chloroform melted at 108-110°. Found: C, 45.49; H, 6.08; O, 48.44; C-CH_3, 6.82; neutralization equivalent, 66.0. Calc. for $C_5H_8O_4$: C, 45.46; H, 6.10;

O, 48.44; 1 C-CH_3, 11.35; neutralization equivalent, 66.05. The compound was found to be identical by mixed melting point and infrared spectrum with authentic methylsuccinic acid (I). The best yield in several runs was 0.54 moles, the average yield was about 0.5 moles.

Fraction (b) crystallized from benzene and melted at 90-91° after sublimation in vacuo. Found: C, 58.41; H, 8.34; C-CH_3, 6.13; neutralization equivalent, 137. Calc. for $C_7H_{12}O_3$: C, 58.38; H, 8.40; 1 C-CH_3, 10.4; neutralization equivalent, 144. Treatment of the acid with diazomethane gave an oily ester which showed in the infrared a hydroxyl peak and an ester peak at 1725 cm^{-1}. The acid may be assigned the structure II. Lithium aluminum hydride reduction of the methyl ester gave an oily diol which took up one mole of periodic acid and yielded the methylcyclopentanone III characterized as a 2,4-dinitrophenylhydrazone, m.p. 134.5°. Found: C, 51.91; H, 5.15; N, 19.62; O, 23.34. Calc. for $C_{12}H_{14}N_4O_4$: C, 51.84; H, 5.08; N, 20.16; O, 23.02%. The infrared spectrum of this derivative was superimposable with the spectrum of an authentic sample of racemic 3-methylcyclopentanone-2,4-dinitrophenylhydrazone. The rotation dispersion curve of the natural ketone III showed a negative Cotton effect.

This requires[3] that this compound be assigned the absolute configuration shown in formula III.

The best yield of compound II was 0.46 moles.

Fraction (a) of the silicic acid chromatogram was rechromatographed on alumina. Petroleum ether-benzene (1:1) eluted a liquid γ-lactone which was distilled at 80° in vacuo (outside temp., 1 mm). Found: C, 67.21; H, 9.75; O, 23.41; M.W. (mass spectroscopy)[4], 142. Calc. for $C_8H_{14}O_2$: C, 67.57; H, 9.93; O, 22.51; M.W., 142. The infrared spectrum showed a γ-lactone band at 1785 cm^{-1}. The N.M.R. spectrum indicated the presence of three C-methyl groups.

Since we had already identified isobutyric acid as one of the fragments we assumed that the lactone might have the structure IVa or IVb. The lactone was reduced with lithium aluminum hydride to the oily diol V. The structure V for the diol was rigorously proved as follows. One isomer (m.p. 175°) of methylisopropylsuccinic acid[5] was

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[3] c.f. Carl Djerassi: Optical Rotatory Dispersion, McGraw-Hill Book Co. Inc., New York (1960), p. 103.

[4] We wish to thank Dr. Klaus Biemann, Chemistry Dept., M.I.T., Cambridge, Mass., for the determination of the mass spectrum. Dr. Biemann also suggested on the basis of the mass spectrum a methyl isopropyl structure for this compound.

[5] C. S. Marvel and J. A. Fuller, J. Am. Chem. Soc. 74, 1506 (1952).

esterified with diazomethane and the ester reduced with lithium aluminum hydride. The resulting oily diol was shown to be identical by infrared and N.M.R. spectra with the 'natural' reduction product V. An examination of the hydrolysis mixture of anhydrooxoryanodol prior to hydrogenation revealed the presence of compounds I and II in an undiminished yield.

We next turned our attention to the problem of the genesis of compound II. In principle, there were two possibilities for its formation. These are portrayed in the structures VI and VII. The possibility VI clearly means that the skeleton of compound II is present in anhydrooxoryanodol, the possibility VII envisages the formation of the acid II by a base catalyzed rearrangement of an actual or potential methylcyclohexane dione. We performed the hydrolysis of anhydrooxoryanodol in a deuterated medium (EtOD + D_2O + KOD) and isolated the deuterated compound VIII in which the deuterium atoms are not exchangeable. Compounds VIII and II had identical melting points and did not show any melting point depression on admixture. The infrared spectra of both compounds were very similar. The N.M.R. spectra of the esters of II and VIII showed identical peaks at 6.32 ppm (3H) methoxyl, 7.24 ppm (1H) hydroxyl, 8.98, 9.06 ppm (3H) C-CH_3. The only difference in the two spectra was the multiplet between 7 and 8.78 ppm which had

an area of 7H in II and an area of 3H in VIII. A determination of deuterium in VIII gave 29.1 atom % XSSD (Calc. for $C_7H_8D_4O_3$ 33 atom % XSSD). This clearly shows that acid II is formed from a six-membered ring precursor present in anhydrooxoryanodol.

Deuterated methylsuccinic acid I was also isolated. It has a deuterium content of 31.58 atom % XSSD and the N.M.R. spectrum of the dimethyl ester showed that the C-methyl group was completely unlabeled. The methyl group was furthermore split into a triplet due to deuterium in the α position. This finding proves rigorously that the methyl group of (I) had been originally present in ryanodol and not created in the basic hydrolysis. The yields of I and II in five runs were almost precisely complementary to one mole. Since, however, a theoretical yield is exceedingly improbable, it seems that the two fragments are not formed from the same carbons. In any case, it is not possible to visualize a mode of formation of I and II in which these two compounds would have more than one single carbon in common. Formally, IV and I could originate from the same carbon atoms. A precursor of the fragment IV could lose acetone and yield a precursor of the fragment I. However, this is exceedingly improbable as the sum of the yields of I and of isobutyric acid clearly exceeds one mole. It would be necessary to assume a second geminal dimethyl group

in ryanodol and this does not seem possible. Consequently, it appears that most of the carbon atoms of ryanodol have been characterized in the fragments I, II and IV.

It is clear that ryanodol is a diterpenoid of a quite unusual type. In order to incorporate the known skeletal elements into a plausible diterpenoid biogenesis, one has to perform a considerable number of rearrangements and/or ring openings, or one has to cyclize the diterpenoid chain in a quite unusual manner.

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