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The Complete Structure of Ryanodine

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The structure of the insecticide ryanodine, $C_{25}H_{35}O_9N$, a constituent of *Ryania speciosa* Vahl.[1], has presented a considerable challenge because of a large number of rings and oxygen substituents and a consequent complexity of reactions on chemical degradation. We now wish to report evidence which leads to the solution of this problem.

It has been known for some time[1] that ryanodine is an ester of the alcohol ryanodol, $C_{20}H_{32}O_8$, and pyrrol-α-carboxylic acid. Recently, we have shown[2] that anhydroryanodine, $C_{25}H_{33}O_8N$, a product of acid treatment or sublimation of ryanodine, has the structure and relative and absolute configuration I, while anhydroryanodol, $C_{20}H_{30}O_7$, formed on a similar treatment of ryanodol, possesses structure II. To complete the solution, the dehydration reaction must now be clarified.

For the structure of ryanodol, the following facts are pertinent:

(1) Ryanodol contains no free carbonyl group (IR);

(2) Ryanodol contains six hydroxyl groups (CH_3OD exchange);

(3) Ryanodol contains no $>C=C<$ (NMR, IR, UV).

Thus, there are five rings in ryanodol, of which two contain oxygen atoms and three are carbocyclic.

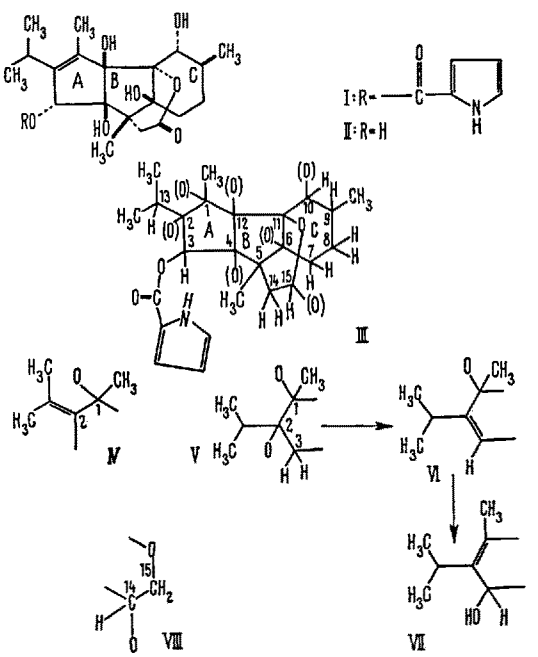
It now remains to establish (a) whether the dehydration involves a skeletal rearrangement, (b) the sites of oxygen attachment, and (c) the nature of the oxygen rings. It follows from the degradation studies described below that ryanodine contains the same carbon skeleton as anhydroryanodine; for the sake of simplicity, this conclusion is anticipated in the use of formula III as an incomplete expression for ryanodine. This formula contains one more oxygen than ryanodine; this is because two of the oxygens in brackets must represent one and the same oxygen atom forming the second hetero ring.

The oxygen substitution in ryanodol follows conclusively from the structure of anhydroryanodol (II) and from the following:

(1) Oxidation of ryanodol with periodate-permanganate yielded isobutyric and α-methylglutaric acid, showing the absence of oxygen atoms at C_7-C_9 and at C_{13} and indicating oxygen substitution at C_2, C_6 and C_{10}.

(2) The NMR-spectra of ryanodol and several of its derivatives show the presence of five methyl groups; of these, three appear as doublets (those located at C_9 and C_{13}) and two as sharp singlets. One of the singlet methyl groups is at about 9.0 τ (C_5), while the second one is normally at about 8.6 τ (C_1). This shows oxygen substitution at C_1.

(3) Treatment of ryanodine with thionyl chloride yielded an amorphous chlorocompound which on vigorous basic hydrolysis gave isoryanodol, $C_{20}H_{32}O_8$ (vide infra). The formation of acetone on ozonolysis of isoryanodol and the NMR-spectrum (vide infra) clearly show the presence of grouping IV in isoryanodol and confirm the presence of an oxygen function at C_2 in ryanodol.



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

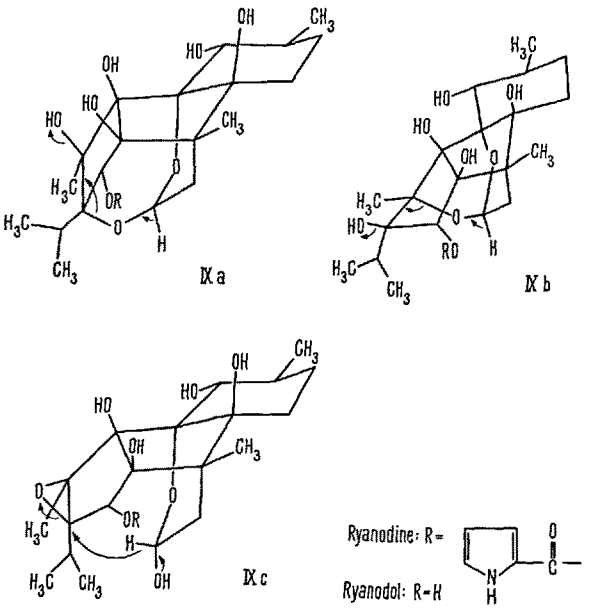
[2] D. R. Babin, T. P. Forrest, Z. Valenta, and K. Wiesner, Exper. *18*, 549 (1962). For a summarizing review of the arguments leading to the structure and absolute stereochemistry of anhydroryanodine see K. Wiesner, *Pure and Applied Chemistry* (1963), p. 285.

(4) The NMR-spectrum of ryanodol in deuterated pyridine clearly shows the presence of three secondary oxygen functions (C*H*–O–) with signals at 5.6 τ, doublet (1 H), 4.9 τ, singlet (1 H), and 4.5 τ, multiplet (1 H). It is clear (see point 1, above) that only four positions – C_3, C_{10}, C_{14} and C_{15} – are available for these three functions. The presence of an oxygen at C_3 (singlet at 4.9 τ) follows conclusively from the structure of anhydroryanodine and anhydroryanodol. The possibility of an allylic rearrangement of the type V→VI→VII during the dehydration is eliminated by the finding that no O^{18} is incorporated into the molecule when anhydroryanodol is prepared in H_2O^{18}. The fact that the C_3–H signal is a sharp singlet confirms oxygen substitution at C_2 and C_4. The periodate-permanganate oxidation (see point 1, above) and the structure of anhydroryanodol show the presence of an oxygen function at C_{10}. This function is clearly visible in the NMR-spectra of various ryanodol and anhydroryanodol derivatives and can be recognized by the position (5.6 τ in pyridine; 6.0–6.2 τ in $CDCl_3$) and large coupling constant (doublet; 10–12 c.p.s.; 1,2-diaxial H's) of the α-H atom. There must, furthermore, be an oxygen function at C_{15} in ryanodol in order to explain the NMR-signal at 4.5 τ for 1 H. The only remaining alternative, VIII, can be easily excluded, since it does not explain why this signal appears at a low-field and since it would furthermore show two additional deshielded hydrogen atoms (C_{15}).

It follows from this analysis that there is no oxygen function at C_{14}. This conclusion is confirmed by the finding that the NMR signal of the C_{14} methylene group is distinctly observable in the spectra of several ryanodol derivatives (vide infra).

The partial formula III must now be completed to the full ryanodine structure by closing an ether ring using two of the bracketed oxygens. It is clear that, on the basis of the above structural definition, the conversion of ryanodine to anhydroryanodine I by an acid-catalysed rearrangement involves a reduction of ring A at the expense of the potential aldehyde function at C_{15} which is simultaneously oxidized to a lactone. *A careful analysis of the problem shows that only for the ryanodine structures* IXa, IXb *and* IXc *can this process be formulated by a reasonable mechanism.* The dehydration pathway portrayed by arrows in formulae IXa and IXb is to our knowledge unprecedented but does not appear unreasonable. The formulae IXa and IXb thus cannot be dismissed on mechanistic grounds[3].

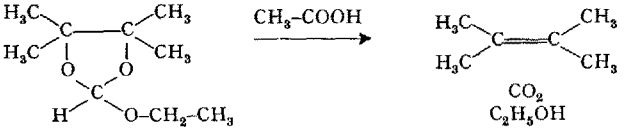
The mechanism of the anhydro reaction for the formula IXc is a more usual one. It is represented by the arrows in IXc. Acid catalysed opening of the epoxide ring creates a carbonium ion at C_2 and the oxido-reduction between this carbon and C_{15} is mediated by a shift of a hydride from C_{15} to C_2. Inspection of a model reveals that the spatial disposition of the groups involved is very suitable for such a process. The formation of anhydroryanodine I is then completed by an acid catalysed elimination of the C_1 hydroxyl formed by the epoxide opening.



We shall now describe several degradative sequences which corroborate the skeletal structure and oxygen substitution of ryanodine and enable us to make a tentative choice between the structures IXa, b and c.

Treatment of ryanodol with periodic acid in water results in the direct crystallization from the reaction mixture of monosecoryanodol, $C_{20}H_{30}O_8$, m.p. 230°, IR (KBr pellet) 1729, 1678 cm^{-1}; UV (EtOH) inflexion 250 mμ (log ε = 2.5), λ_{max} 332 mμ (log ε = 2.14); NMR, no aldehyde or methyl ketone signal. On the basis of the structural alternatives IXa, b and c for ryanodine, structures Xa, b and c have to be considered for this compound[4]. Furthermore, if formula IXc should turn out to be correct for ryanodine, it becomes necessary to consider also structure Xd for monosecoryanodol. With the opening of the AB ring system, the transoid five-membered hemiacetal group becomes possible and

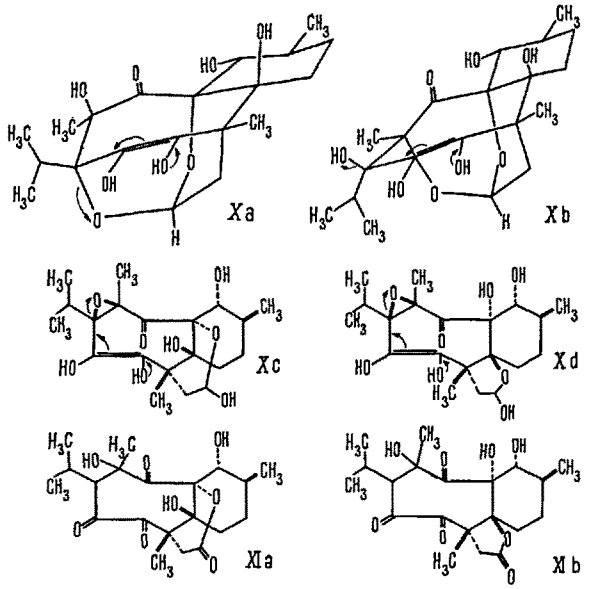
[3] A certain formal analogy to the postulated mechanism is the reaction



described recently. (G. CRANK and F. W. EASTWOOD, Aust. J. Chem. *17*, 1392 (1964).)

[4] The formulation of an enol at C_3–C_4 in structures Xa, b, c, d is based on IR-spectra. The question whether monosecoryanodol is an enol or the corresponding ketone does not affect the interpretation of reactions described below.

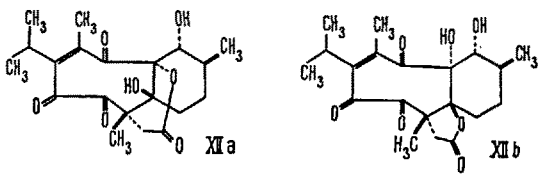
the position of the equilibrium between the five-membered and six-membered hemiacetal is not easy to estimate.



Oxidation of monosecoryanodol with chromic acid in 90% aqueous acetic acid at low temperature yielded a monosecolactone, $C_{20}H_{28}O_8$, m.p. 207°, IR (KBr pellet) 1785, 1748, 1711 cm^{-1}; UV (EtOH) inflexion 240 mμ (log $\varepsilon = 2.87$), λ_{max} 294 mμ (log $\varepsilon = 2.42$). As shown by subsequent reactions and the NMR-study described below, this compound is formed by an elimination of the C_2 oxygen function and an oxidation of the hemiacetal group which in Xc and d is already present and which in the structure Xa is formed in the elimination reaction. The acetal is not opened by elimination in formula IXb; here, it is necessary to assume an acid catalysed acetal opening as an independent process, preceding the oxidation.

The monosecolactone must consequently be formulated as XIa or b[5]. The IR-spectrum of the monosecolactone favours structure XIb. This, however, does not mean that formula Xd may be automatically adopted for monosecoryanodol. The structural possibilities Xa and Xb could have suffered the cleavage of the acetal group and a transformation of the six-membered to the five-membered hemiacetal prior to oxidation to the lactone; thus, they could also yield the monosecolactone represented by XIb.

Acetylation of the monosecolactone with acetic anhydride and pyridine yielded a well-defined diacetate, $C_{24}H_{32}O_{10}$, m.p. 252°. The NMR-spectrum of this derivative is particularly revealing. The signal at the lowest field is a doublet at 4.7 τ corresponding to the C_{10} hydrogen deshielded by an acetoxyl; this is the only hydrogen deshielded by oxygen in the spectrum. In addition, the spectrum shows two acetyl methyls at 7.8 τ and 7.9 τ, the C_1 methyl deshielded by oxygen as a singlet at 8.4 τ, and the four remaining methyl groups as a singlet and three doublets between 8.8 τ and 9.1 τ. The C_{14} methylene group is seen as a singlet at 6.75 τ.



When lactone XI is melted at 215° and then sublimed, a crystalline anhydro compound is obtained, $C_{20}H_{26}O_7$, m.p. 228°, IR 1802, 1778, 1650, 1598 cm^{-1}; UV $\lambda_{max}^{EtOH} = 298$ mμ (log $\varepsilon = 4.24$). Structure XIIa or XIIb follows automatically for this anhydro compound from the structures XIa or b for the monosecolactone. The UV-spectrum of this compound is anomalous, but it is considered that the possible UV anomalies in a system like XII are not predictable. On the other hand, the NMR-spectrum of XII corroborates the structure to a considerable extent.

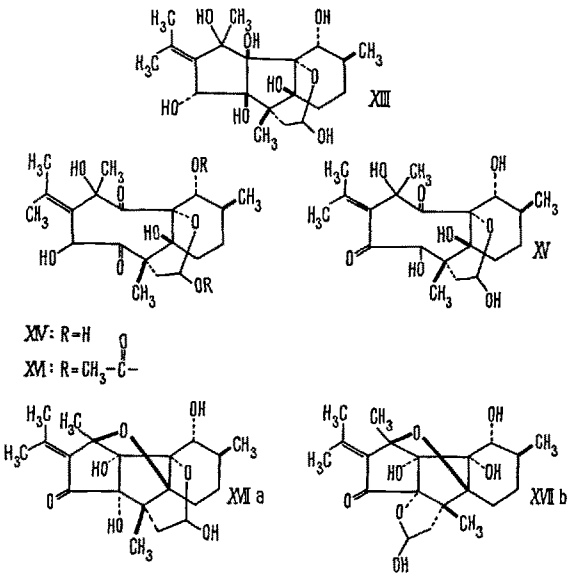
It shows the usual doublet at 5.98 τ for the hydrogen at C_{10}, a quadruplet centred at 7.2 τ for the C_{14} methylenic group, a singlet at 8.36 τ for the C_1 methyl and, finally, a singlet and three doublets for the four remaining methyl groups between 8.8 and 9.1 τ. It should be pointed out that the presence of these characteristic signals is perhaps not more important for the corroboration of structure XII than is the absence of any signals, except the C_{10} hydrogen, in the low-field portion of the spectrum.

Treatment of ryanodine at $-5°$ with thionyl chloride in pyridine followed by hydrolysis of the chlorine-containing product with alcoholic alkali gave a crystalline compound, isoryanodol, $C_{20}H_{32}O_8$, m.p. 261°, IR no carbonyl group; UV end absorption; NMR singlet (6 H) 8.23 τ (isopropylidene group), singlet (3 H) 8.66 τ (C_1 methyl group deshielded by oxygen), singlet and doublet (6 H) above 8.90 τ (remaining two methyl groups). The presence of the isopropylidene group was confirmed by ozonolysis which yielded acetone. Formula XIII can be assigned to isoryanodol regardless of the question which of the ryanodol structures IXa, b or c is correct.

Isoryanodol rapidly consumed one mole of periodic acid and yielded isooxoryanodol which could not be induced to crystallize. However, the precipitated product gave a good analysis for $C_{20}H_{30}O_8$, IR 1751, 1682 cm^{-1}; UV strong end absorption with a shoulder at

[5] It should be noted that the configuration of C_1 in the lactone derived from Xb is opposite to that derived from Xa, c and d.

265 mμ. Isooxoryanodol may be represented as XIV with possibly a small amount of the isomerized product XV present.



Acetylation of isooxoryanodol yielded a well-defined crystalline diacetate, $C_{24}H_{34}O_{10}$, m.p. 184°, IR 1763, 1700 cm^{-1}; UV end absorption, to which we assign the structure XVI. Its NMR-spectrum is in good agreement with this formulation; it contains a broad unresolved multiplet (1 H) at 3.95 τ (C_{15} hydrogen deshielded by hemiacetal acetate), a doublet (1 H) at 4.59 τ (C_{10} hydrogen deshielded by acetate), a singlet (1 H) at 5.4 τ (C_3 hydrogen deshielded by hydroxyl), singlets (3 H each) at 7.82, 7.94, 8.47, 8.90, a singlet (6 H) at 8.02 and a doublet (3 H) at 9.1 τ.

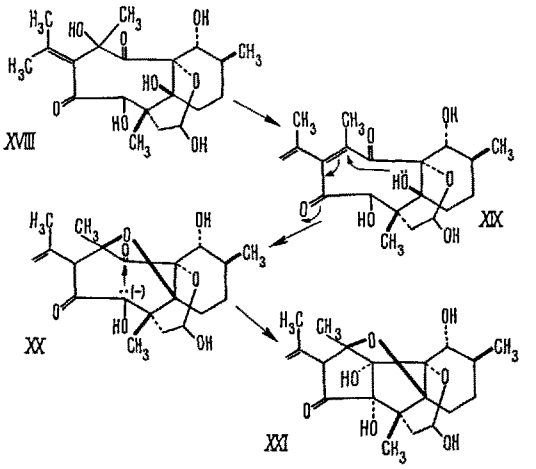
When isooxoryanodol XIV is treated with dilute sodium carbonate solution, a new crystalline anhydro compound is obtained, $C_{20}H_{28}O_7$, m.p. 228°, IR 1708, 1620 cm^{-1}; UV $\lambda_{max}^{EtOH} = 270$ mμ (log $\varepsilon = 3.9$). Formula XVIIa or b can be assigned to this compound.

The NMR-spectrum corroborates certain features of formula XVII. It shows a doublet (1 H) at 6.18 τ for the C_{10} hydrogen while the signal for the hydrogen at C_{15} is obscured since the spectrum had to be recorded in deuterated methanol. Two singlets (3 H each) are located at 7.75 and 7.97 τ; these correspond to the isopropylidene group and their relative position to the C_3 ketone is proved by their disappearance on equilibration with alkaline deuterated methanol. Singlets (3 H each) are located at 8.52 and 9.17 τ, corresponding to the ring A and ring B methyl group, respectively, and a doublet (3 H) of the ring C methyl group appears at 9.0 τ.

The transformation of XIV to XVII is very simple and may be represented by the sequence

XIV→XVIII→XIX→XX→XXI→XVIIa

which is self-explanatory.

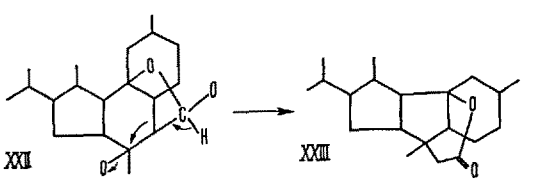


In the course of any stage in this sequence a change of the six-membered to a five-membered hemiacetal can take place yielding structure XVIIb. Compound XVII yields a well-defined crystalline diacetate, $C_{24}H_{32}O_9$, m.p. 258°. The NMR-spectrum of this derivative shows that the two secondary hydroxyl groups have been acetylated; the C_{15} hydrogen appears as an unresolved multiplet (1 H) at 3.89 τ and the C_{10} hydrogen as a doublet (1 H) at 5.22 τ.

In all our attempts to oxidize the hemiacetal function to a lactone, cleavages of the vicinal diol systems resulted. Consequently, advantage was taken of the stability of isoryanodol XIII to acid and a crystalline diacetonide of this compound, $C_{26}H_{40}O_8$, m.p. 245°, was prepared by treatment with *p*-toluenesulfonic acid and acetone. The diacetonide was oxidized smoothly by bromine in water buffered with barium carbonate to the corresponding lactone, $C_{26}H_{38}O_8$, m.p. 245°, IR 1745 cm^{-1} (lactone); UV end absorption, which crystallized with a molecule of methanol. The NMR-spectrum showed the presence of the hydrogen deshielded by the ring C hydroxyl as a doublet (1 H) centred at 6.12 τ.

The reactions described so far corroborate two presumptions:

(1) The skeleton of ryanodine is identical with the skeleton which we have previously deduced for anhydroryanodine; anhydroryanodine was therefore not generated by a skeletal rearrangement of the type XXII → XXIII.



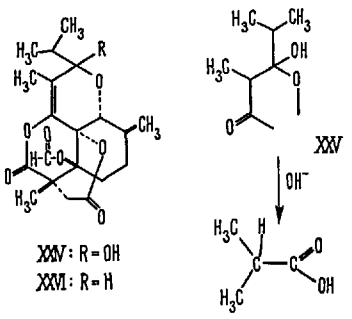
(2) The oxygen distribution in the ryanodine skeleton is correctly shown in partial structure III. Mecha-

nistic considerations (vide supra) of the anhydro rearrangement then enable us to develop the partial structure III to IXa, IXb or IXc and all three of these ryanodine formulae are in reasonable agreement with the data presented so far.

The only finding which could be interpreted as favoring the hemiacetal structure IXc is the distinct acidity of ryanodol. Continuous ether extraction of ryanodol from a solution of aqueous alkali is a process which requires several weeks; if, however, carbon dioxide is introduced into the solution, the extraction is complete in 6 h. The direct proof of the presence of a hemiacetal function in ryanodol by oxidation does not succeed without cleavage of the vicinal diol system, and when such a cleavage occurs the formation of a lactone is no longer diagnostic (vide supra). The protection of the vicinal diol system also does not succeed, since, in acidic medium, ryanodol undergoes the anhydro rearrangement with great facility.

It seemed to us that a definition of the differences between the formulae IXa, b and c might be best achieved by an interpretation of the reaction of ryanodol with three moles of periodate which yields a crystalline product, $C_{20}H_{26}O_8$[6]. After all, the differences between IXa, b and c are exactly of the kind to influence profoundly the course of this reaction. As it turned out, we have been able to deduce and corroborate unambiguously the structure XXIV for the oxidation product. Unfortunately, XXIV is again derivable from all three formulae IXa, b, c and constitutes a very strong corroboration of them. However, it will be seen that, mechanistically, *the formation of* XXIV *from* IXc *is a favoured process.*

The preparation and properties of this compound have already been described[6]. In the IR-spectrum, XXIV shows a single sharp hydroxyl band at 3595 cm^{-1}, a broad very strong carbonyl band between 1780 and 1740 cm^{-1} assigned to the enol lactone, δ lactone and formate and a sharp band at 1695 cm^{-1} assigned to the enol lactone double bond.



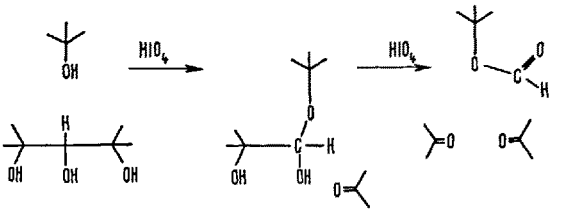
However, it is the already reported NMR-spectrum of XXIV[6] which is particularly revealing. It shows a singlet (1 H) at 1.93 τ for the formate hydrogen and a doublet (1 H) at 6.12 τ which is assigned to the C_{10} hydrogen. This hydrogen and the adjacent methyl group are thus proved to be completely undisturbed. A further low-field signal which turns out to be extremely important *by its clear absence* is the signal for the C_{15} hydrogen. A careful consideration of the matter reveals that the only mechanism by which this hydrogen could have disappeared is an oxidation of a hemiacetal to a lactone. This conclusion is immediately corroborated by the presence of a quadruplet (2 H) centred at 7.2 τ and assigned to the C_{14} methylene group. The methyl group signals are also in excellent agreement with formula XXIV. A singlet (3 H) at 8.18 τ is assigned to the vinylic methyl group while the C_5 methyl deshielded by the enol lactone appears as a singlet at 8.6 τ. Finally, three doublets (9 H) between 8.91 and 9.19 τ are assigned to the isopropyl and ring C methyls. Inspection of structure XXIV reveals that the compound contains the potential grouping XXV. In agreement with this, the compound was found to generate quantitatively isobutyric acid with dilute alkali. This experiment also verified the assignment of the 1.93 τ singlet to a formate ester since formic acid was simultaneously produced.

Finally, the presence of an allylic hemiketal in XXIV was rigorously proved as follows. Hydrogenation of XXIV with platinum oxide in methanol yielded the desoxy compound $C_{20}H_{26}O_7$, m.p. 204°. The IR-spectrum of this compound is essentially the same as that of compound XXIV, but it contains *no hydroxyl peak.* It is clear that the only hydroxyl of XXIV, contained in the allylic hemiketal, has been hydrogenolytically replaced by a hydrogen. Consequently, we formulate the hydrogenolysis product as XXVI. Now, if the hydrogenolytically replaced hydroxyl was indeed a hemiketal hydroxyl, the NMR-spectrum of XXVI must show a new signal for a hydrogen unshielded by oxygen. This is in fact observed. The NMR-spectra of XXIV and XXVI are essentially the same except for the presence, in the spectrum of XXVI, of a narrow doublet (1 H) at 5.9 τ. Thus, practically all the features of XXIV are proved and if we give due consideration to the known structure of anhydroryanodol, the entire formula XXIV is secure.

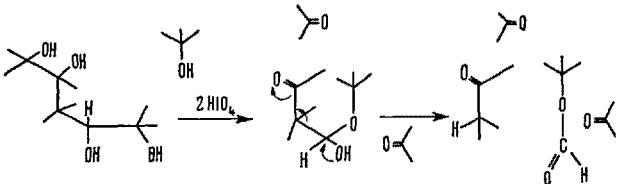
It is now necessary to derive structure XXIV in an unexceptionally plausible manner from one of the ryanodol formulae IXa, b or c. We begin this process by deducing the origin of the formate group in XXIV. Clearly, a formate ester may originate from a hemiacetal group by either a periodate cleavage with a vicinal hydroxyl or a cleavage reaction with a carbonyl or leaving group in the β position. Since none of these

[6] D. R. Babin, J. A. Findlay, T. P. Forrest, F. Fried, M. Götz, Z. Valenta, and K. Wiesner, Tetrahedron No. 15, 31 (1960).

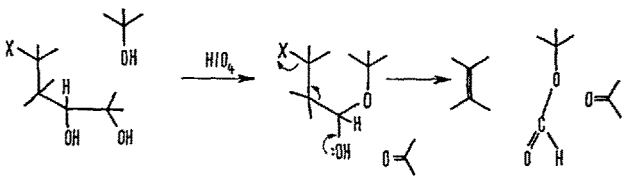
requirements can be met by the actual or potential hemiacetal function at C_{15} in IXa, b or c, this carbon may be excluded as the source of formate. A second possibility for the genesis of the formate ester is a secondary hydroxyl. Such a group may give rise to a formate ester by the methods illustrated in the schemes A, B and C.



Scheme A

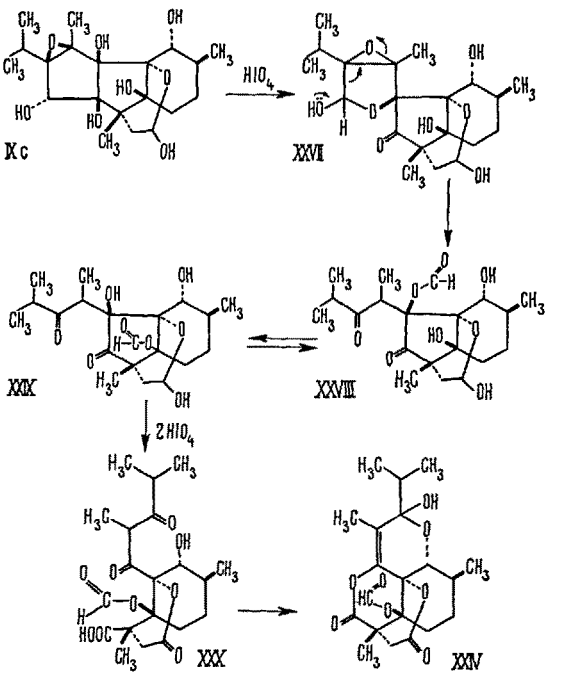


Scheme B



Scheme C

Inspection of the two secondary hydroxyls in the ryanodol structures IXa, b, c reveals that the ring C hydroxyl cannot be the source of the formate since it does not possess the required environment and since it follows from the NMR-spectrum of XXIV that the ring C hydroxyl did not participate in any cleavages. It is thus clear that only the hydroxyl at C_3 can generate the formate and an examination of the three methods A, B and C with the three possible ryanodol structures IXa, b and c reveals that *only the application of method* C *makes it possible to deduce a formula for the oxidation product which possesses all of the proved features, i.e. the formula* XXIV. This may be done formally with all three structures IX, but it will be noted that only structure IXc possesses a leaving group X, i.e. one terminus of an epoxide, commonly considered capable of an easy ejection. Consequently, we illustrate the formation of XXIV using the ryanodol structure IXc, but we have to admit that IXa and b are not yet excluded with complete certainty.



The scheme

IXc → XXVII → XXVIII → XXIX → XXX → XXIV

represents the pathway proposed for the formation of XXIV from ryanodol on oxidation with periodic acid.

Zusammenfassung. Die Struktur des Ryanodins und die Natur der Ryanodin-Anhydroryanodin-Umlagerung wird diskutiert.