THE STRUCTURE, STEREOCHEMISTRY AND ABSOLUTE CONFIGURATION OF ANHYDRORYANODINE

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The insecticidal principle ryanodine was isolated from the flacourtiaceous plant *Ryania speciosa* Vahl by Folkers and his collaborators[1]. The powdered stems of this plant are being used in New Brunswick for the control of the European corn borer and we became interested in this problem chiefly because of the ready availability of the plant material, which was donated to us in unlimited quantities by S. B. Penick and Company, New York.

Ryanodine $C_{25}H_{35}O_9N$ suffers a very easy dehydration either on mild acid treatment or on sublimation. The resulting compound, anhydroryanodine $C_{25}H_{33}O_8N$, lends itself much better to degradation studies than ryanodine itself. Because of this fact and since the two compounds must be in a relatively simple relationship, we have decided to first elucidate the structure of anhydroryanodine. This objective has now been accomplished and forms the subject of the present lecture.

The study of ryanodine was initiated at the University of New Brunswick in 1951. At that time, we had established that ryanodine was an ester of pyrrole-α-carboxylic acid and of ryanodol $C_{20}H_{32}O_8$.

According to analysis, active hydrogen determination and the absence of carbonyl groups in the infrared spectrum, ryanodol appeared to be a pentacyclic compound with six hydroxyls and two ether bridges. On treatment with acid, ryanodol was found to suffer a dehydration analogous to the dehydration of ryanodine and to yield anhydroryanodol $C_{20}H_{30}O_7$. This dehydration was connected with the appearance of a carbonyl group in the infrared spectrum at 1721 cm^{-1}, which was later shown to be due to a δ-lactone group. Anhydroryanodol consumed one mole of periodate and yielded anhydro-oxoryanodol $C_{20}H_{28}O_7$. This compound was found to be extremely labile to alkali and disintegrated on basic treatment into a number of acids, one of which was identified as isobutyric acid. At this stage these preliminary findings were reported[2], and the problem was temporarily abandoned since the small New Brunswick group was fully occupied with the structure elucidation of the first garrya and aconitum alkaloids.

When the study of ryanodine was resumed in 1960 the chances of achieving a rapid solution of the problem were substantially better. The department has, in the meantime, acquired a nuclear magnetic resonance spectrograph, but, more important, we have gained experience in the study of polyfunctional

polycyclic compounds which gave us confidence in the ultimate resolution of ryanodine chemistry. A careful study of various ryanodol derivatives by n.m.r. spectroscopy revealed five C-methyl groups, of which two were, of course, part of the isopropyl known to be present (see fragmentation, *Figure 1*).

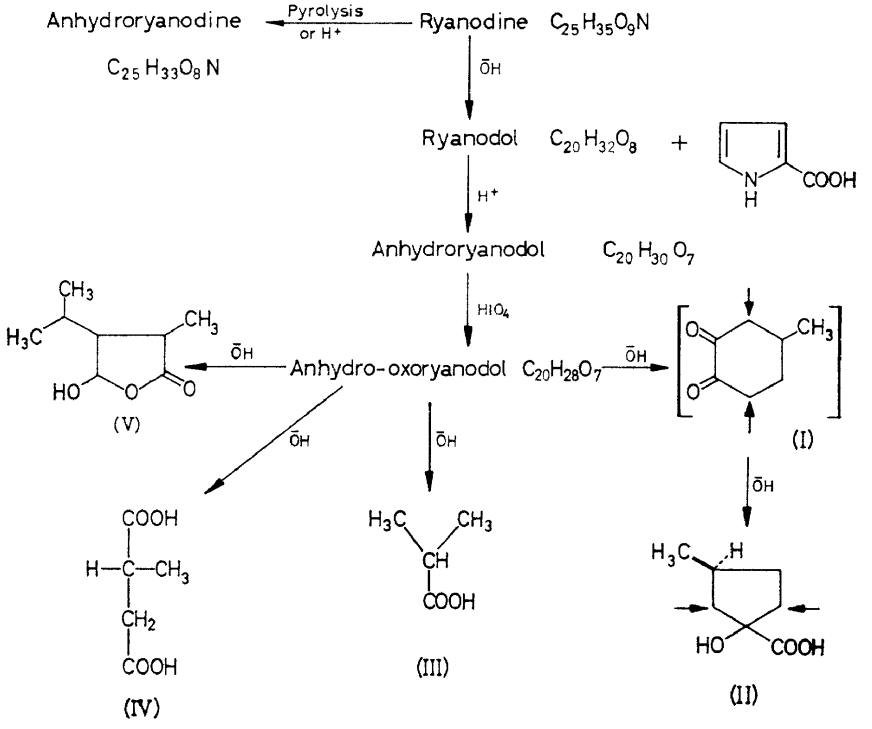


Figure 1

The functionality of ryanodol was further clarified when the presence of six hydroxyls was corroborated by careful deuteration studies and when it was shown that one of the two ether bridges and a hydroxyl were part of a hemiacetal group. This conclusion followed *inter alia* from the finding that several periodate cleavage products of ryanodol (diseco and triseco-ryanodol) were formate esters. The hemiacetal group in ryanodol enabled us also to understand in a preliminary fashion the genesis of the δ-lactone group in the anhydro reaction. Anhydroryanodol revealed in the n.m.r. spectrum a fully substituted double bond which carried one of the five methyl groups. The formation of an orthoacetate diacetate which possessed no hydroxyl and the presence of the aforementioned δ-lactone group accounted for all the oxygen.

We next turned to the identification of the fragmentation products obtained by basic treatment of oxoanhydroryanodol. The fragmentation products were separated by partition chromatography on silicic acid with water as the stationary phase and chloroform with a varying percentage

of butanol as the moving phase. The structures of the individual compounds were elucidated by very simple methods[3] which do not require a detailed description and are portrayed in *Figure 1* (formulae (II)–(V)).

It was possible to demonstrate that compound (II) originated from a benzilic acid rearrangement of the intermediate cyclohexandione (I).

In compound (II) the two methylenic groups marked by arrows are non-exchangeable for deuterium in an alkaline deuterated medium. In the intermediate (I), on the other hand, such an exchange reaction is possible. We have, therefore, performed the fragmentation reaction in heavy water and obtained the acid (II) fully deuterated in the two marked methylenic groups—a decisive proof for the origin of compound (II) from a six-membered ring.

By lithium aluminium hydride reduction of the methyl ester of (II) a glycol was obtained which on periodate cleavage yielded (−)-3-methyl-cyclopentanone of known absolute configuration. This finding later became the basis of the absolute configuration assignment of anhydroryanodine.

The yields of all compounds in the fragmentation were such that no overlap of any of the products except (III) and (V) was possible and, consequently, all carbon of anhydroryanodol was accounted for. At this stage we came to the conclusion that while the fragmentation scheme probably holds the key to the complete structure of anhydroryanodol, it would require an extremely lucky idea to deduce the structure from these data alone. In fact, we have speculated on the basis of a (incorrect) biogenetic hypothesis derived from the fragmentation scheme and arrived at structures closely related to the right one but never completely correct even in skeleton.

Consequently, we decided that we needed an idea experiment, for instance a dehydrogenation result, to help us orientate the fragments with respect to each other. After some unsuccessful dehydrogenation studies, we turned to the treatment of anhydroryanodol with refluxing hydroiodic acid and red phosphorus. This reaction gave a mixture of products from which the γ-lactone $C_{20}H_{26}O_2$ (VI) was isolated in good yield. A clue to the structure of (VI) was found in an uncharacterized amorphous fraction from the same experiment which gave on selenium dehydrogenation a hydrocarbon $C_{19}H_{22}$ formulated as (VII). Compound (VII) was clearly a fluorene by its ultraviolet spectrum and showed in the n.m.r. spectrum four aromatic methyls (7·40, 7·60, 7·68 and 7·77 ppm) and two benzylic methyls (singlet (6H) at 8·63 ppm). The n.m.r. spectrum also showed four aromatic hydrogens at 2·4–3·0 ppm. In view of the common origin of (VI) and (VII) one methyl group was placed on ring " C " and three on ring " A ".

Since in compound (VI) the absence of a substituent in the position marked by an arrow was proved (by degradation to compound (XI)), it was necessary to distribute the three methyls on ring " A " in (VII) as shown, to account for the relationship of (VI) and (VII) by a relatively simple Wagner rearrangement[4].

The n.m.r. spectrum of (VII) and the infrared spectrum of (VI) considered together enabled us also to place the aromatic methyl in ring " C ". The infrared of (VI) was in clear agreement with the presence of a 1,2,4 substituted benzene ring. Since in compound (VII) the hydrogen coming to resonance at the lowest field must be the one marked by the asterisk

(unshielding effect of the aromatic ring " A ") and since the lowest peak in the n.m.r. spectrum of (VII) is a singlet we were able to select the 1,2,4 substitution shown and discard the second possible alternative.

The structural studies performed on (VI) are too lengthy to be recounted in detail. Suffice it to say that they included the conversion of (VI) into

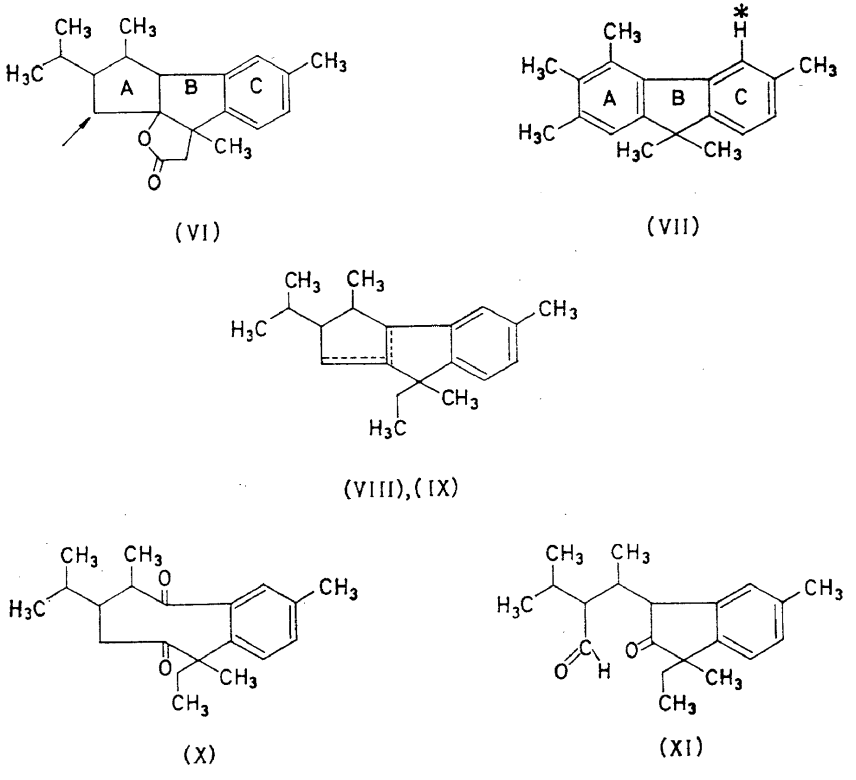


Figure 2

the two unsaturated isomers (VIII) and (IX) and the two seco derivatives (X) and (XI) by standard methods. A preparative Kuhn-Roth oxidation of (VI) gave a mixture of acetic and isobutyric acid. All these data would still permit an exchange in the positions of the methyl and isopropyl group in ring " A ". The alternative chosen has been rigorously proved to exist in anhydroryanodol.

The entire N.M.R. spectrum of compound (VI) is in excellent agreement with the structure, and the assignments of the various peaks are shown in *Figure 3.*

Of course, there was no assurance at this stage that in the formation of (VI) extensive rearrangements did not occur. However, we were pleased to see that the structure of compound (VI) may be simply dissected to yield

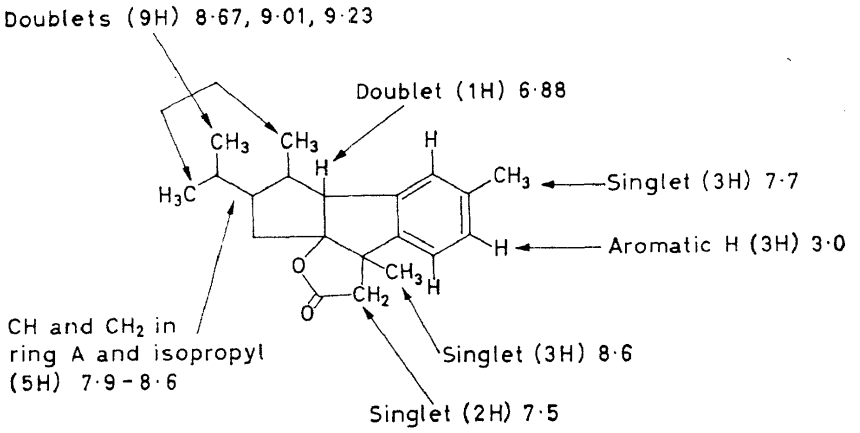


Figure 3

without overlap the skeletons of the fragments obtained in the basic degradation of oxoanhydroryanodol. This is illustrated in *Figure 4*.

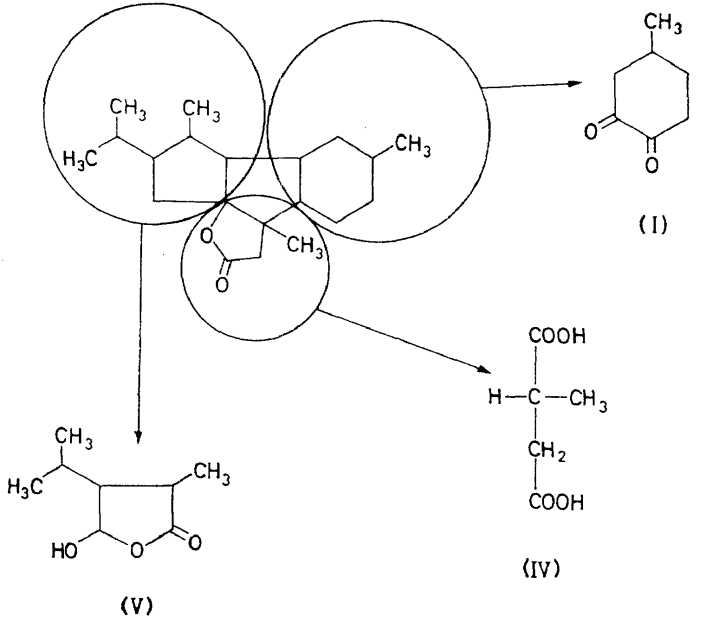


Figure 4

Studies of functional groups and their environment in anhydroryanodol which we performed at this point increased the areas of known skeletal identity between anhydroryanodol and the lactone (VI) to such an extent that we finally accepted the identity of the entire skeleton of the two compounds as extremely probable. A particularly useful derivative in this

respect was anhydroryanodol orthoacetate diacetate (XII). This compound was hydrolysed by mild treatment with alkali to an orthoacetate monoacetate which in turn was oxidized to the fully substituted cyclopentenone (XIII). These transformations are portrayed in *Figure 5.*

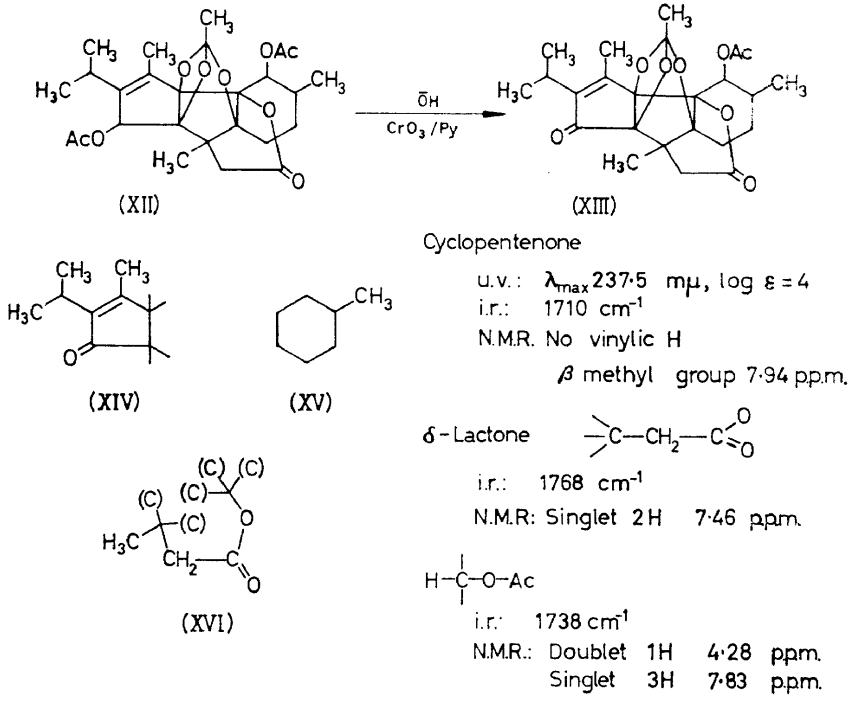


Figure 5

The spectroscopic assignments of the various structural elements are given in the figure and do not require any comment. The i.r. frequency of the δ-lactone group is high due to strain caused by the orthoacetate formation. Anhydroryanodol itself is a typical δ-lactone (i.r. max. 1720 cm^{-1}).

The assignment of the lactone hydroxyl as tertiary and the two acetyl groups as secondary follows from the fact that neither anhydroryanodol nor the orthoacetate (XII) with both acetyl groups removed by hydrolysis show any hydrogen unshielded by a lactonic hydroxyl in the n.m.r. spectrum.

It may now be easily seen that the presence of the elements (XIV), (XV) and (XVI) is proved in compound (XIII). The presence of the isopropyl group in the α-position of the cyclopentenone is not only to be expected on the basis of the structures of lactone (VI) and the methyl isopropyl γ-lactol (V), but follows from the fact that the α-position is not occupied by hydrogen or methyl (n.m.r.) and also not by a ring junction (violation of the Bredt rule). Consequently, the isopropyl group is the only group present in anhydroryanodol eligible to occupy this position. The proof of the presence

of the element (XV) in anhydroryanodol has been already discussed in connection with the fragmentation reaction. Finally, the acetic acid side chain (XVI) which forms the δ-lactone is attached (n.m.r.) to a quaternary carbon. By exclusion, it becomes clear that this structural element must be the source of methyl succinic acid in the fragmentation reaction. Consequently, we must place a methyl group as indicated in (XVI). The objection that the methyl group in methyl succinic acid may not be derived from a methyl of anhydroryanodol, but from a methylenic group by breaking of a bond, is easily met by the already-mentioned fragmentation experiment run in heavy water. The methyl succinic acid isolated from this experiment contained no deuterium in the methyl group. If the methyl group was derived from a fragmentation the intermediate in this reaction would have picked up at least one deuterone from the medium.

The structural elements (XIV), (XV) and (XVI) may now be assembled into very few complete skeletal structures, one of which is identical with the skeleton of lactone (VI). It may be shown by a careful analysis of all of them, that only this last one can explain all the chemistry and especially provide us with a simple and unexceptionally plausible explanation of the fragmentation reaction.

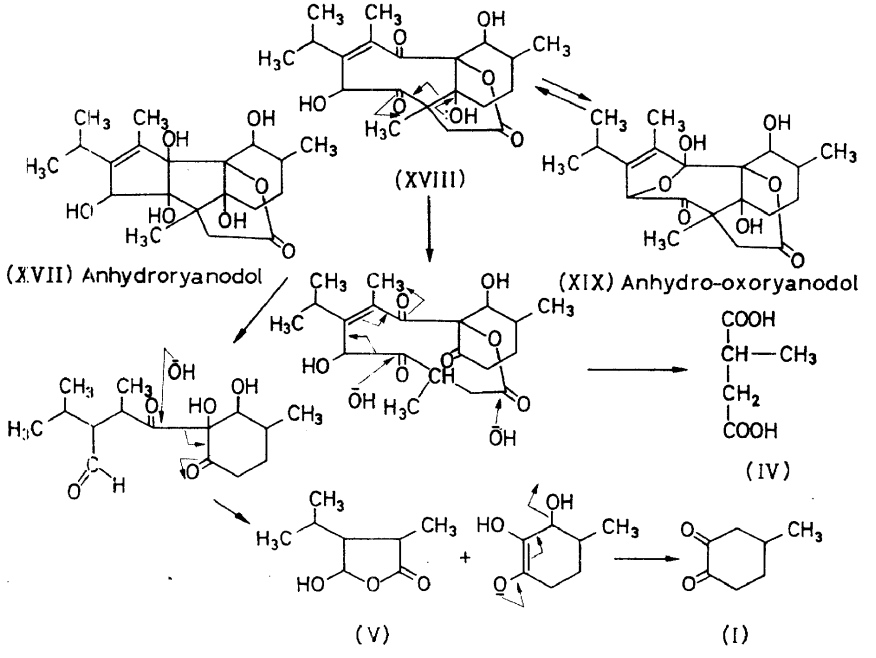
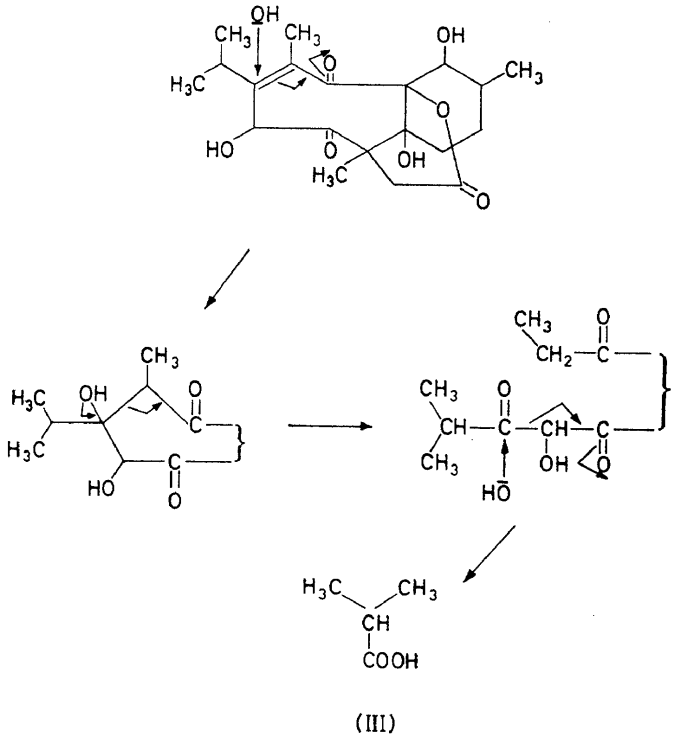


Figure 6

If anhydroryanodol is (XVII) (*Figure 6*), we may represent anhydro-oxoryanodol as (XIX) with a masked α,β-unsaturated keto group. This masking explains both the absence of a u.v. chromophore and a sharp uptake

of only one mole of periodate by (XVII). The base-catalysed fragmentation may then be rationalized by the simple and unexceptional schemes shown in *Figures 6* and *7* which do not require any comment.



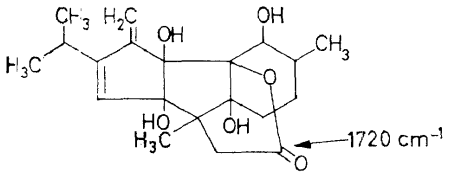
(III)

Figure 7

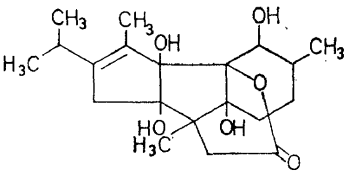
For the further corroboration of the anhydroryanodol structure (XVII), the bis-anhydro series is of importance. Bisanhydroryanodol (XX) (*Figure 8*) is obtained by treatment of ryanodol with 20 per cent sulphuric acid at 90°. It yields one mole of formaldehyde on ozonolysis and consumes one mole of periodate.

Hydrogenation of bisanhydroryanodol yields the dihydro derivative (XXI). Both (XX) and (XXI) may be converted to the corresponding orthoacetates monoacetates. The identity of the skeletal structure of anhydro- and bisanhydroryanodol is proved by the facile conversion of anhydroryanodol orthoacetate monoacetate to the orthoacetate monoacetate of (XX) by thionyl chloride in pyridine. If the orthoacetate monoacetate of (XXI) represented by (XXII) is reduced by lithium aluminium hydride the triol (XXIII) is obtained. Cleavage of this compound by one mole of lead tetra-acetate yields the tertiary aldehyde (XXV) (n.m.r. singlet (1H) 0·53 ppm) by an aldol condensation of the primary cleavage product (XXIV). This sequence proves clearly that the former lactone hydroxyl, the ring " C " hydroxyl and the ring " C " methyl group are located on adjacent carbons.

With the structure of anhydroryanodol determined the only remaining structural problem was the location of the pyrrole-α-carboxylic residue in anhydroryanodine. It was possible to solve this question very simply in the following manner. Anhydroryanodine was converted into the orthoacetate

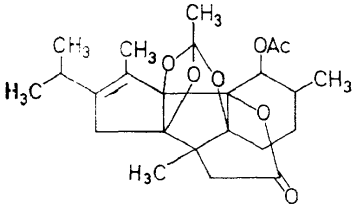


(XX)

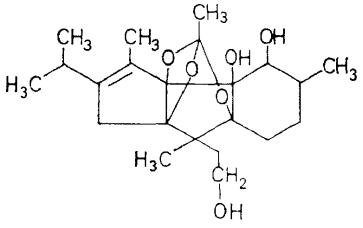


(XXI)

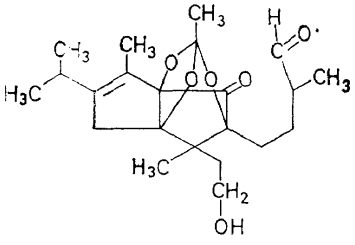
u.v. $\lambda_{max.}$ 235·5 (log ε = 4·17)



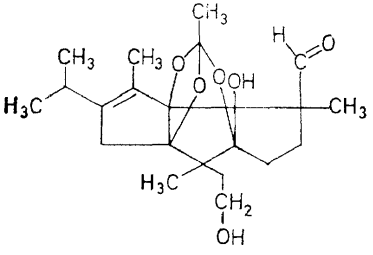
(XXII)



(XXIII)



(XXIV)



(XXV)

Figure 8

diacetate (XXVI). This was at first puzzling, since anhydroryanodine must possess one less free hydroxyl than anhydroryanodol (*i.e.* four hydroxyls). However, it was soon realized from the changed ultraviolet absorption that one acetyl group has entered the pyrrole nucleus. The same chromophore is obtained if methylpyrrole-α-carboxylate is subjected to the identical acetylation procedure.

We have next tried to hydrolyse selectively the allylic ester (as we have done before in the case of anhydroryanodol orthoacetate diacetate) to determine the identity of the acyl group esterified to the allylic hydroxyl. This turned out to be impossible, since the new acetyl-pyrrole-α-carboxylic ester group was sufficiently acidic to dissociate completely to an anion in

dilute alkali and thus render the ester group immune to hydrolysis. Compound (XXVI) was, therefore, methylated with diazomethane and converted into the non-acidic *N*-methyl derivative. This compound was not characterized, but it hydrolysed smoothly to anhydroryanodol orthoacetate monoacetate (XXVII) which has been previously shown to possess a free allylic hydroxyl in ring " A " (by oxidation to the cyclopentenone (XIII)).

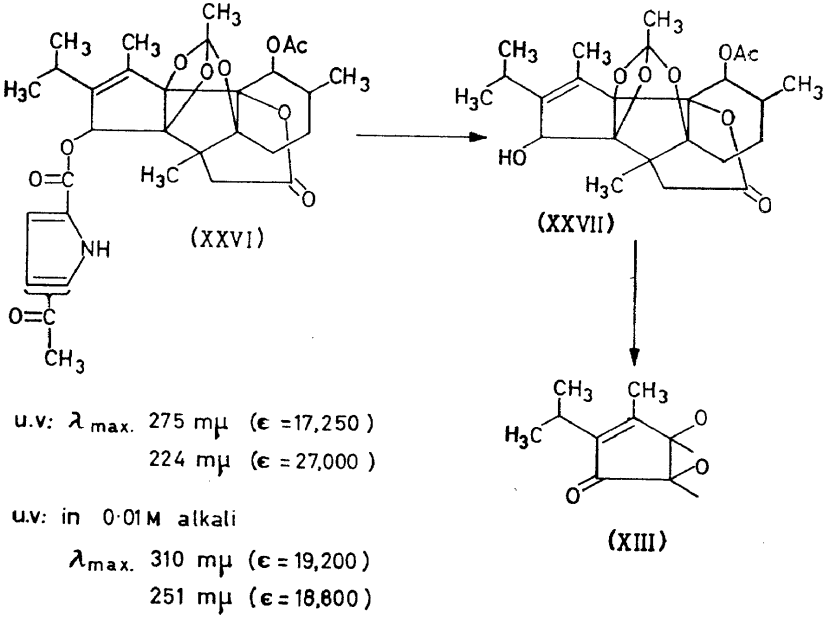


Figure 9

The stereochemistry and absolute configuration of anhydroryanodine turned out to be a relatively simple problem. It was possible to show[5] without any difficulties and with the use of already available experimental data that anhydroryanodine must be represented by the complete stereo structure (XXVIII) shown in *Figure 10.*

The argument is best started with the absolute configuration of the ring " C " methyl group. This configuration is known, since this particular asymmetric carbon was isolated in the form of the methylcyclopentanone (XXX) obtained from oxoanhydroryanodol *via* the cyclohexandione (XXIX (*Figure 10*).

The ring " C " hydroxyl must be trans to the methyl group since in many n.m.r. spectra of various anhydroryanodine derivatives, the hydrogen unshielded by this hydroxyl is found as a sharp doublet with a separation of 8·5–10·5 c/s. Such a splitting shows clearly that the hydrogen in question and its neighbour must be *trans* diaxial. The configuration of the remaining asymmetric centres follows automatically from the limitations imposed on

the system by the lactone group and by the formation of the orthoacetate derivatives.

The only exception is the configuration of the allylic hydroxyl in ring " A ". This is represented as *trans* to the free α-diol group to explain the uptake of only one mole of periodate by anhydroryanodol. The ditertiary *cis*-diol reacts faster, and further cleavage is prevented by the hemiketal formation between one keto group and the allylic hydroxyl.

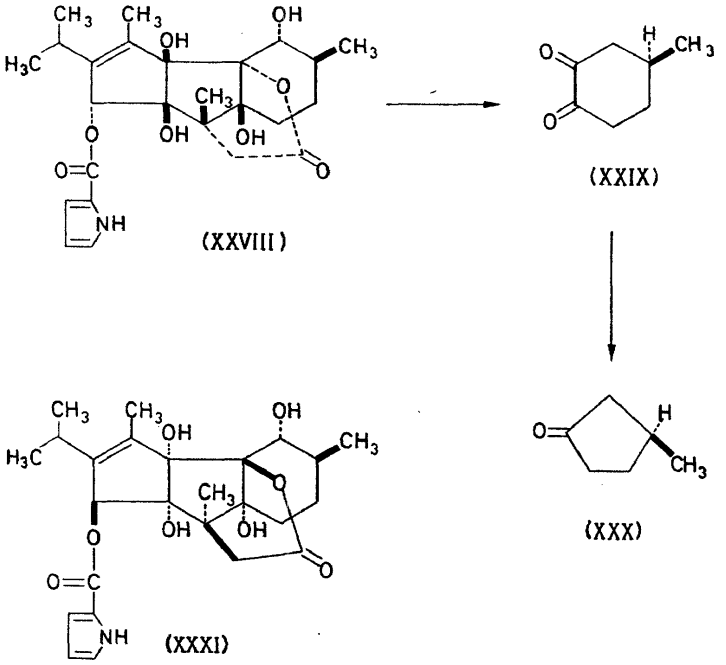


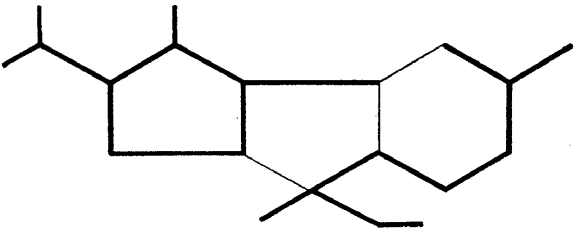
Figure 10

This assignment is now corroborated by some n.m.r. spectroscopic observations. All anhydroryanodol derivatives possessing in some form the allylic hydroxyl show in the n.m.r. spectrum the methylenic group adjacent to the lactone carbonyl as a quadruplet.

On the other hand, all derivatives where the allylic hydroxyl is removed and replaced by a double bond or a methylene group or a keto group show the methylene adjacent to the lactone carbonyl as a singlet.

Such an influence of the allylic hydroxyl on the unequal shielding of the two hydrogens in question is only possible if the configuration is as postulated in (XXVIII). The only other stereochemical alternative for anhydroryanodine is portrayed in the formula (XXXI). It suffers from the disadvantage, that it would be necessary to assume the boat form as the preferential conformation of ring " C ", to explain the observed splitting displayed by the hydrogen unshielded by the ring " C " hydroxyl.

As we have stated before, the relationship between the structure of ryanodine and anhydroryanodine must be a relatively simple one and consequently, it is perhaps not premature to consider at this point a possible ryanodine biogenesis. We can see (*Figure 11*, formula (XXXII)) that the



(XXXII)

Figure 11

skeleton is a combination of a cyclopentanoid monoterpene and a regular terpenoid chain. The mode of junction of these two moieties is unusual but it seems likely that ryanodine is essentially a new type of a polysubstituted diterpene.

I am greatly indebted to a group of enthusiastic colleagues and students whose efforts, persistence and ability are largely responsible for the success of these investigations.

The first phase of the project was carried out with (Dr) R. B. Kelly, Dr M. Špaček and Dr D. J. Whittingham. The second far more extensive period of our work was conducted as a joint project with my former student, later colleague, and present successor, Professor Zdenek Valenta. The students and post-doctoral fellows who participated in this phase of the work were (Dr) D. R. Babin, (Dr) T. Bögri, (Dr) J. A. Findlay, (Dr) T. P. Forrest. (Dr) F. Fried, Dr M. Götz and Dr H. Reinshagen.

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