cycle oxydo-réducteur et phosphorylant des réactions 2-3-4-5-6-1 (Figure 6) ou 2-3-5′-6′-4′-8 (Figure 8).

Quoi qu'il en soit et pour nous résumer, la nouveauté essentielle de ces mécanismes hypothétiques réside en l'addition 1-4 d'acide phosphorique à la méthylènequinone postulée par CHMIELEWSKA[11] suivie d'une migration du phosphore sur l'oxygène phénolique. Cette façon de voir, qui tient également compte du fait que le méthyle en 2[18] et la double liaison de la chaîne latérale semblent essentielles pour la phosphorylation[3], permet d'interpréter la formation d'un phosphate d'hydroquinone à partir du phosphate minéral sans incorporation dans le noyau quinonique d'oxygène venant du milieu[19,20].

Summary. Two possible mechanisms for oxidative phosphorylation are suggested, based on participation of quinones in the process.

Both of them postulate the 1-4 addition of inorganic phosphate on a reactive quinone isomer (the quinone-methide II) without exchange of the quinone oxygen atoms. They also account for the P_i-$^{18}OH_2$ exchange observed during oxidative phosphorylation.

M. VILKAS et E. LEDERER

Laboratoire de Chimie de l'Ecole Normale Supérieure, Paris, et Laboratoire de Chimie Biologique, Faculté des Sciences de l'Université de Paris (France), le 23 juillet 1962.

[18] La plastoquinone est dépourvue de méthyle en 2. Si elle participe aux phosphorylations oxydatives, ce qui ne semble pas encore prouvé avec certitude, elle le fait par un mécanisme différent de ceux envisagés ici pour les méthyl-2 quinones, à moins que le méthyle en 6 puisse jouer le même rôle que celui qui manque en 2.

[19] Nous remercions les Collègues qui ont bien voulu discuter de ce problème avec nous, qu'ils partagent ou non notre point de vue: E. RACKER, MLLE B. TCHOUBAR, Lord TODD et R. B. WOODWARD.

[20] Dans deux ouvrages récents L. L. INGRAHAM, *Biochemical Mechanisms* (John Wiley, 1962), p. 47 et E. M. KOSOWER, *Molecular Biochemistry* (McGraw Hill, 1962), p. 21, les auteurs envisagent une estérification directe par l'anion phosphate (éventuellement complexé par Mg^{++}) de l'anion d'une hydroquinone ou d'une semi-quinone, donc sans échange de l'oxygène quinonique.

The Complete Structure and Relative and Absolute Configuration of Anhydroryanodine

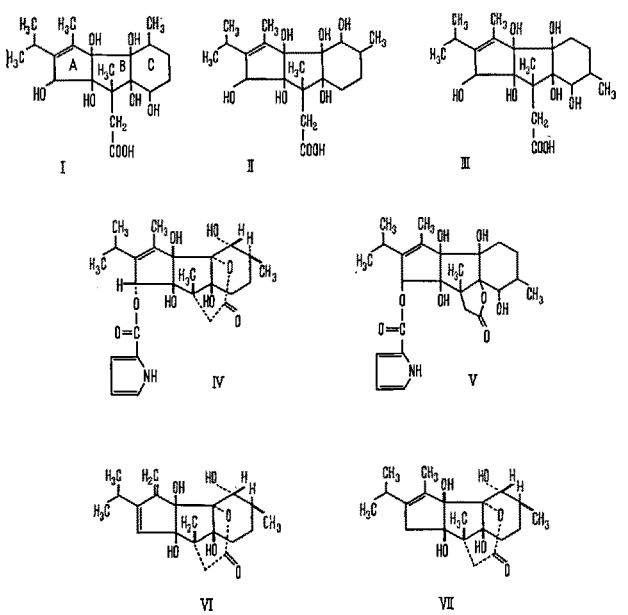
In a recent preliminary note[1], it has been shown that anhydroryanodol must be represented by one of the structures I, II, and III in which the carboxyl is lactonized to an unspecified hydroxyl group. We now wish to extend the argument and propose the complete structure, configuration, and absolute configuration IV for anhydroryanodine, the dehydration product of ryanodine, easily obtainable either on sublimation or acid treatment of the latter compound[2]. A second possibility which is not in accord with part of our evidence but cannot, at the present time, be excluded rigorously, is represented in V.

The location of the pyrrole-α-carboxylic acid residue in anhydroryanodine was determined as follows: Anhydroryanodine was acetylated with acetic anhydride-perchloric acid at −10°C. This reaction gave a crystalline product, $C_{31}H_{37}O_{10}N$ (m.p. 259°C; IR 1735, 1675 cm^{-1}; UV, $\lambda_{max} = 275$ mμ ($\varepsilon = 17250$), 224 mμ ($\varepsilon = 27000$), shoulder 250 mμ ($\varepsilon = 11600$); UV (0.01 *M* alcoholic KOH), $\lambda_{max} = 310$ mμ ($\varepsilon = 19200$), 251 mμ ($\varepsilon = 18800$)). The analysis indicates that the product is an *ortho*-acetate diacetate. The ultraviolet spectrum shows clearly that one of the acetyl groups has entered the pyrrole nucleus, presumably in the 4 or 5 position. The same chromophore may be obtained if the identical acetylation procedure is applied to methyl pyrrole-α-carboxylate.

Since this chromophore forms an anion in alkaline solution (as shown by the UV-spectra), it was decided to methylate it with diazomethane in order to convert it into a nondissociating group; this should then be amenable to a differential saponification under mild conditions. This expectation was indeed fulfilled; the alkaline hydrolysis of the uncharacterized methylated product yielded the previously described[1] anhydroryanodol *ortho*-acetate monoacetate (VIII), which has been shown[1] to possess a free allylic hydroxyl in ring A. It is now clear that this hydroxyl must have been the site of attachment of the pyrrole-α-carboxylic acid residue.

For the further development of the anhydroryanodine structure, the bis-anhydro series is of importance. Bis-anhydroryanodol, $C_{20}H_{28}O_6$, (VI) (m.p. 235°C; IR, 1725, 1637, 1618 cm^{-1}; UV, $\lambda_{max} = 235.5$ mμ ($\log\varepsilon = 4.17$)) may be obtained by treating ryanodol at 90° with 20% sulphuric acid. It is also, in addition to anhydroryanodine, one of the chief products isolated from a high vacuum sublimation of ryanodine.

Compound VI gave formaldehyde on ozonolysis and consumed one mole of periodic acid. Hydrogenation gave the dihydroderivative VII ($C_{20}H_{30}O_6$, m.p. 204°C; IR, 1725 cm^{-1}; UV, end absorption). The N.M.R. spectra of VI and VII are in agreement with the proposed location of the double bonds. Both compounds VI and VII yield the



[1] Z. VALENTA and K. WIESNER et al., Exper. *18*, 111 (1962).

[2] R. B. KELLY, D. J. WHITTINGHAM, and K. WIESNER, Can. J. Chem. *29*, 905 (1951).

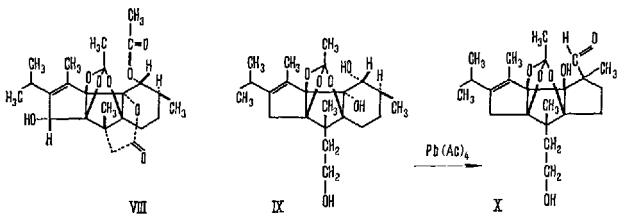
corresponding *ortho*-acetate monoacetates ($C_{24}H_{30}O_7$; m.p. 207°C; IR, 1753, 1643, 1593 cm^{-1}; UV, λ_{max} = 232.5 mμ (log ε = 4.2); $C_{24}H_{32}O_7$; m.p. 210°C; IR, 1761, 1731 cm^{-1}; UV, end absorption). These *ortho*-acetates are now assumed to be unrearranged derivatives of VI and VII. The higher wave-numbers of the lactone peaks are presumably caused by an increased strain introduced by the *ortho*-acetate group.

The absence of any further rearrangement as well as the location of the diene system in the *bis*-anhydro series is made probable by the quantitative conversion of anhydroryanodol *ortho*-acetate monoacetate[1], now formulated as VIII, into *bis*-anhydroryanodol *ortho*-acetate monoacetate by thionyl chloride in pyridine.

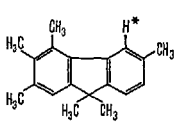
The attachment of the lactone hydroxyl at a carbon adjacent to the ring C hydroxyl was proved as follows. Dihydro-*bis*-anhydroryanodol *ortho*-acetate monoacetate was reduced by lithium aluminum hydride to the triol IX, $C_{22}H_{34}O_6$, m.p. 191°. This compound took up one mole of lead tetraacetate and yielded the oily aldehyde X, formed by an aldol condensation of the intermediate keto-aldehyde. Compound X clearly showed an aldehyde singlet in the N.M.R. spectrum at 0.53 ppm. This finding indicates that the former lactone hydroxyl, the ring C hydroxyl and the ring C methyl group are located on adjacent carbons.

In many N.M.R. spectra of various ryanodine derivatives, the hydrogen unshielded by the ring C hydroxyl is found as a sharp doublet with a separation of 8.5–10.5 cycles/sec. This splitting shows that the interacting hydrogens are diaxial *trans*; consequently, the ring C hydroxyl and methyl must be 1, 2-*trans* diequatorial. The location of the methyl group on ring C is indicated by the structure of the previously described hexamethylfluorene[1] which is now regarded to be XI. This assignment is made on the basis of the fact that the aromatic hydrogen which is expected to resonate at the lowest field (H*) is a singlet at 2.4 ppm.

Since the absolute configuration of the ring C methyl is known[3], the entire absolute and relative configuration of anhydroryanodine follows automatically from the structure assignment IV. The only point of uncertainty is the configuration of the acyl group in ring A. It is assumed to be *trans* to the free α-diol group to explain the uptake of only one mole of periodate or lead tetraacetate by anhydroryanodol derivatives.

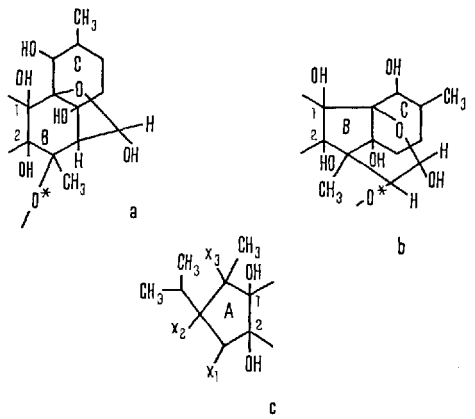


VIII IX X



XI

The complete structure of anhydroryanodine imposes severe limitations on the structure of ryanodine itself. Considerations about the mechanism of the anhydro reaction allow only those structures from the previously published general scheme for ryanodine[1] which have rings B and C constituted as in Formula a. However, ryanodine structures possessing the anhydro skeleton (partial Formula b) which have been previously dismissed on the basis of N.M.R. evidence, cannot be ruled out rigorously. The same applies to structures which contain a secondary oxygen function in ring A and therefore do not require an allylic rearrangement during the formation of anhydroryanodine. N.M.R. evidence (the number of hydrogens deshielded by oxygen atoms) seems to rule them out, but the interpretation of N.M.R. spectra in the ryanodine series is too difficult to be considered rigorous.



a b c

Consequently, the formation of the anhydro-compounds limits the structure of ryanodine to a combination of the system c with either a or b. In the partial structure c, one of X_1, X_2, and X_3 is a hydrogen atom. The remaining two substituents X together with the asterisked oxygen in structure a or b account for the attachment of pyrrol α-carboxylic acid and an ether ring. In this scheme, several ethers are sterically implausible, while formulae with the pyrrol α-carboxylate at X_2 do not allow a rational formulation of the anhydro reaction. This leaves a total of 11 structures for ryanodine.

The involvement of an allylic rearrangement in the anhydro reaction as well as the size of ring B in ryanodine are under active study[4].

Zusammenfassung. Für Anhydroryanodin wurde Struktur IV bewiesen.

D. R. BABIN, T. P. FORREST, Z. VALENTA, and K. WIESNER

Organic Chemistry Laboratory, University of New Brunswick (Canada), June 19, 1962.

D. R. BABIN, J. A. FINDLAY, T. P. FORREST, F. FRIED, M. GÖTZ, Z. VALENTA, and K. WIESNER, Tetrahedron Letters *15*, 31 (1960).

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