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The Constitutions of Glauconic, Glaucanic and Byssochlamic Acids

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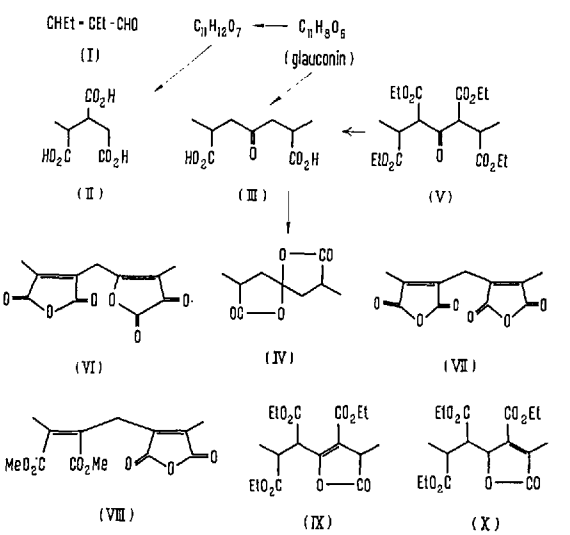
Glauconic and glaucanic acids were first isolated by WIJKMAN[1] from a mould, described as *Penicillium glaucum*, which has since been reclassified as *Penicillium purpurogenum*[2]. The chemistry of glauconic acid, $C_{18}H_{20}O_7$, was extensively studied at München by WIJKMAN, KRAFT, SUTTER et al.[1,3–7] who showed that it contained an acylable hydroxyl group and titrated as a tetra-carboxylic acid. Reduction with zinc dust and acetic acid gave a dihydro-derivative to which we make further reference below. The main effort[1,3–7] of the earlier workers was concentrated on the pyrolysis products of glauconic acid. On heating to 200° the molecule breaks down into diethylacrolein[3] (I), $C_7H_{12}O$, identified as the corresponding acid[5], and glauconin, $C_{11}H_8O_6$. This latter compound was studied in some detail.

Glauconin titrated as a tetracarboxylic acid and on ozonolysis gave more than one mole of pyruvic acid[4] as well as oxaloacetic acid[4]. One of the most informative experiments was the reduction of glauconin with red phosphorus and hydriodic acid to a dihydro-derivative, isolated as a compound of the composition ($C_{11}H_{10}O_6 + H_2O$). The latter was, in fact, a dicarboxylic acid, which, on conversion to the dimethyl ester with diazomethane and ozonolysis, gave (after hydrolysis of the product) the tricarboxylic acid (II). The constitution of this tricarboxylic acid was confirmed by synthesis[7].

When glauconin was heated with hydrochloric acid at 200° it afforded a mixture of the meso- and racemic forms of the keto-dicarboxylic acid (III) as well as the three possible racemates of the dilactone (IV). The keto-dicarboxylic acid (III) was synthesised[4] by double alkylation of acetonedicarboxylic ester with ethyl α-bromopropionate to give the tetra-ester (V). Hydrolysis of the latter gave the desired keto-diacid (III) which with acetyl chloride cyclised to the dilactone (IV). Mainly on the basis of this evidence the constitution (VI) was proposed for glauconin. Although the alternative (VII) has been considered, and rejected, by the earlier workers[7] it appeared to us to explain the chemistry of glauconin better than (VI). In the event the correctness of the formula (VII) has been conclusively established by spectral evidence and by synthesis.

The ultraviolet spectrum of glauconin in *non-hydroxylic* solvents showed $\lambda_{max}^{cyclohexane}$ 250 mμ ($\varepsilon = 10200$) indicative of two dialkylmaleic anhydride residues. The infrared spectrum showed two five-membered anhydride rings. This conclusion was confirmed by inspection of the infrared spectrum of glauconin dimethyl ester (VIII), a compound obtained by methylating glauconin with dimethyl sulphate under alkaline conditions[6]. The N.M.R. (nuclear magnetic resonance) spectrum[8] of glauconin in tri-



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[1] N. WIJKMAN, Liebigs Ann. *485*, 61 (1931).

[2] Personal communication from Mr. G. SMITH, London School of Hygiene and Tropical Medicine: see J. L. YUILL, Biochem. J. *28*, 222 (1934).—M. TAKASHIMA, A. KITAJIMA, and K. OTSUKA, Chem. Abstr. *52*, 20379d (1958).

[3] H. SUTTER and N. WIJKMAN, Liebigs Ann. *505*, 248 (1933).

[4] H. SUTTER and N. WIJKMAN, Liebigs Ann. *519*, 97 (1935).

[5] H. SUTTER, F. ROTTMAYR, and H. PORSCH, Liebigs Ann. *521*, 189 (1936).

[6] K. KRAFT and H. PORSCH, Liebigs Ann. *527*, 168 (1937).

[7] K. KRAFT, Liebigs Ann. *530*, 20 (1937).

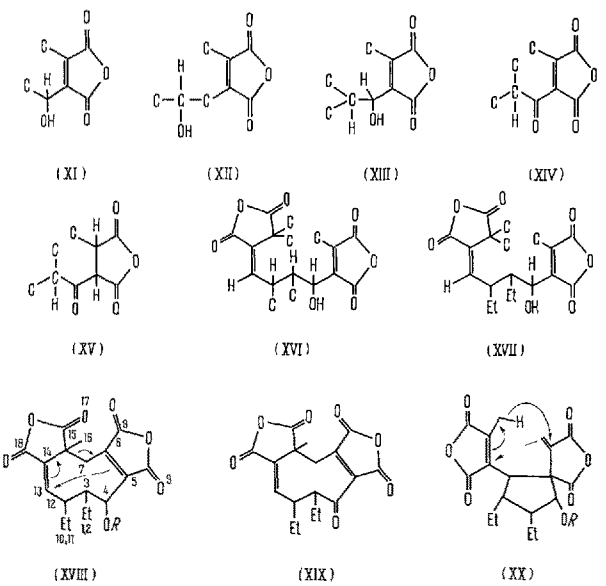
[8] All signments of N.M.R. bands are based on L. M. JACKMAN, *Nuclear Magnetic Resonance Spectroscopy* (Pergamon Press, London 1959).

fluoroacetic acid showed the presence of two identical unsplit methyl groups ($\tau = 7.70$) attached to double bonds. The other two protons showed a single peak at $\tau = 6.26$ in agreement with the presence of a methylene group flanked by two ethylenic linkages.

The constitution of glauconin as (VII) was confirmed by synthesis according to the following method. The known tetra-ester (V) (see above) was converted by refluxing in benzene in the presence of methanesulphonic acid (azeotropic removal of water and ethanol) into a mixture of the lactones (IX) and (X). The enol-lactone was the main component of the mixture. On alkaline hydrolysis it afforded the keto-dicarboxylic acid (III). The second ester (X) gave, by β-elimination, prototropic migration and hydrolysis, glauconin (VII) identical (m.p., mixed m.p., ultraviolet and infrared spectra) with authentic material. The synthesis is essentially a reversal of the hydrochloric acid induced degradation of glauconin to the keto-dicarboxylic acid (III).

We now turn to the constitution of glauconic acid, $[\alpha]_D + 33°$ (c, 1.02), λ_{max} 223 mμ ($\varepsilon = 10500$), inflexion near 260 mμ, ν_{max} 3510, 1830, 1770, and 1670 cm^{-1}. It can be seen that the infrared spectrum confirms the presence of an hydroxyl group and suggests that the molecule contains one or more cyclic five-membered anhydride functions probably conjugated with ethylenic linkages. The presence of two anhydride systems is conclusively established by derivatives described in the sequel. Using the Δ^1- and Δ^2-tetrahydrophthalic anhydrides as models for the possible chromophores formed from cyclic anhydrides and ethylenic linkages, it was found that the ultraviolet absorption spectrum of glauconic acid was best accounted for by the presence of one Δ^1-type chromophore ($\lambda_{max}^{cyclohexane}$ 250 mμ ($\varepsilon =$ 3540)) and one Δ^2-type chromophore ($\lambda_{max}^{cyclohexane}$ 223 mμ ($\varepsilon = 7600$)). The ultraviolet spectra of various derivatives (see below) have confirmed this conclusion.

As briefly noted above, reduction of glauconic acid with zinc dust in refluxing acetic acid gives[4] a dihydro-derivative, $[\alpha]_D + 57°$ (c, 1.01), λ_{max} 230 mμ ($\varepsilon = 8000$), ν_{max} 1835, 1767, and 1720 cm^{-1}, which must contain an itaconic type of anhydride chromophore. That this reduction involves more than the saturation of an ethylenic linkage is obvious from the fact that the compound is a carboxylic acid giving, with diazomethane, a monomethyl ester $[\alpha]_D + 34°$ (c, 1.21), λ_{max} 232 mμ ($\varepsilon = 8550$), ν_{max} 1840, 1780, and 1740 cm^{-1}. This methyl ester shows *no* hydroxyl absorption in its infrared spectrum. The simplest rationalisation of these facts is that the maleic anhydride residue has been reduced and the reduced anhydride ring opened with concomitant lactonisation. On treatment with dimethyl sulphate and alkali dihydroglauconic acid gives[7] a trimethyl ester, the anhydride ring being opened and methylated. This ester shows, ν_{max} 1775 and 1745 cm^{-1}, indicating the presence of a γ-lactone grouping as well as of the three ester residues. The part-formulae (XI) and (XII) will explain this transformation. The former (XI) is preferred for two reasons. First, reduction of glauconic acid acetate[1], $[\alpha]_D + 35°$ (c, 1.20), λ_{max} 221 mμ ($\varepsilon = 10600$), inflexion at about 250 mμ, ν_{max} 1835, 1770, and 1740 cm^{-1}, with zinc dust in refluxing acetic acid gave glaucanic acid, $C_{18}H_{20}O_6$, $[\alpha]_D + 185°$ (c, 1.05), λ_{max} 220 mμ ($\varepsilon = 10700$), inflexion at about 250 mμ, ν_{max} 1840 and 1775 cm^{-1}. As would be expected from theoretical considerations developed later, glaucanic acid itself is reduced to a dihydro-glaucanic acid on more prolonged reduction[6]. Glaucanic acid must be desoxygaluconic acid. The removal of the acetyl residue under such mild conditions is most easily understood if it is allylic to the maleic anhydride type residue[9]. Secondly, the N.M.R. spectrum of glauconic acid acetate shows that there is a hydrogen atom attached to the carbon atom bearing the acetoxyl group and that this is split into a doublet ($\tau = 4.15$; $J = 5$ c.p.s.). There is, therefore, only one proton on the neighbouring (α) carbon atoms[8]. However, in dihydro-glauconic acid methyl ester (see above) this same proton now appears as a triplet ($\tau = 5.06$; $J = 4$ c.p.s.) so that it must be split by *two* protons. Only if we base our constitutional arguments on formula (XI) is there the requisite increase in the number of neighbouring protons on reduction. The change in the position of absorption of the proton is also in agreement with the postulated structural change[8]. The part-formula (XI) can now be expanded to (XIII) on the basis of these arguments.



[9] It will be shown in the sequel that the hydroxyl group of glauconic acid cannot be allylic to the ethylenic linkage of the itaconic anhydride residue.

Glauconic acid can be smoothly oxidised to a *ketone*, $C_{18}H_{18}O_7$, m.p. 174–176°, $[\alpha]_D + 45°$ (c, 0.56), λ_{max} 220 mμ ($\varepsilon = 10000$), inflexion at about 290 mμ, ν_{max} 1830, 1770, and 1695 cm^{-1}, with chromium trioxide in acetone[10]. This ketone is readily reduced under the usual zinc dust-acetic acid conditions to a *keto-tricarboxylic acid*, $C_{17}H_{24}O_7$, m.p. 171–173° (decomp.), $[\alpha]_D - 90°$ (c, 1.00), $\varepsilon_{220\,m\mu} = 600$, ν_{max} 1750 and 1735 cm^{-1}, readily converted by diazomethane into a *keto-trimethyl ester*, $C_{20}H_{30}O_7$, m.p. 106–108°, $[\alpha]_D - 101°$ (c, 1.00), $\varepsilon_{220\,m\mu} = 500$, ν_{max} 1735 cm^{-1}. On heating, the tricarboxylic acid gave an *anhydride*, $C_{17}H_{22}O_6$, m.p. 168–170°, $[\alpha]_D - 59°$ (c, 1.00), $\varepsilon_{220\,m\mu} = 550$, ν_{max} 1860, 1785, 1750, and 1720 cm^{-1}. The absorption in the infrared due to the ketonic carbonyl group is best resolved in this anhydride and indicates (at 1750 cm^{-1}) that the compound is a cyclopentanone. Furthermore, from the lack of ultraviolet absorption it must be concluded that besides reduction of the maleic anhydride type residue and decarboxylation the itaconic anhydride type residue has also disappeared. This can only be explained by cyclisation. A monocyclic substance, the ketone, must therefore have been cyclised to a bicyclic compound. Since the ketonic carbonyl was necessary for this reaction to take place, the most simple explanation was that an intramolecular Michael reaction[11] between one of the position α- to the ketone group and the β-position of the itaconic residue had occurred. When the maleic anhydride residue in the ketone, depicted in (XIV), is reduced, there are two α-positions (on either side of the ketone) which could act as anion sources for the Michael reaction (see (XV)). However, it must be the position between the two carbonyl groups in (XV) which is involved for the following reason. During the reduction of glauconic acid ketone with zinc a second product is formed. This is a *monocyclic anhydride-acid*, $C_{17}H_{22}O_6$, m.p. 170–180°, $[\alpha]_D + 8°$ (c, 1.00), λ_{max} 227 mμ ($\varepsilon = 7100$), ν_{max} 1830, 1770, and 1700 cm^{-1}, characterised as its *methyl ester*, $C_{18}H_{24}O_6$, m.p. 115–117°, $[\alpha]_D \pm 0°$ (c, 1.00), λ_{max} 225 mμ ($\varepsilon = 8300$), $\nu_{max}^{CHCl_3}$ 1830, 1770, 1725, and 1670 cm^{-1}. Obviously this compound retains the itaconic anhydride type residue intact but one carbonyl group has been lost. The maleic anhydride type residue has also been reduced in the normal way. This anhydride-acid does *not* cyclise to the bicyclic derivative under the conditions of the zinc dust-acetic acid reduction. One must conclude therefore that cyclisation precedes the loss of the carboxyl group and that the latter is mandatory for cyclisation. This can only be understood if the 1:3-dicarbonyl system of (XV) (or its hydrated equivalent) is required for the cyclisation. Indeed, decarboxylation may be synchronous with cyclisation. In any case the 'anion' for the cyclisation must be on the carbon atom between the 1:3-dicarbonyl system of (XV). We can now join the maleic anhydride type residue to its itaconic analogue in such a manner that the postulated cyclisation will place the ketonic group in a five-membered ring. The part formula (XIII) can therefore be expanded to (XVI), certain structural conclusions justified in the sequel being also anticipated.

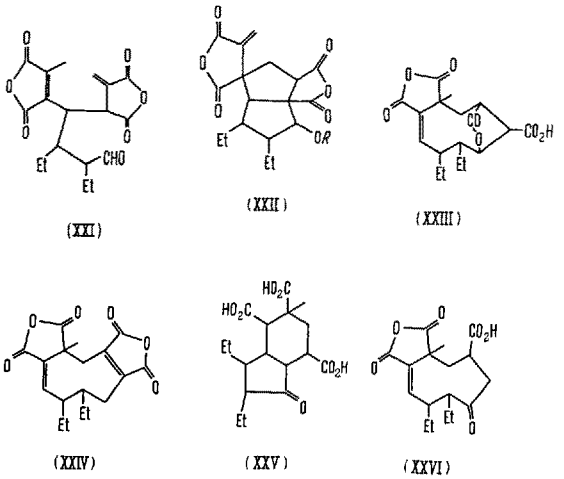
The integrated N.M.R. spectrum of glauconic acid acetate shows the presence of *one* olefinic proton ($\tau = 3.11$; $J = 13$ c.p.s.) split as a doublet. The doublet is retained at the same position in the ketone from glauconic acid. This excludes any formula with the hydroxyl group allylic to the itaconic anhydride type residue. If the latter had been correct, the formation of the ketone would have removed the proton splitting the vinylic proton. The possibility that the proton which is causing the splitting of the vinylic proton is α- to the carbonyl of the itaconic anhydride type residue, i.e., that coupling is taking place across the double bond, is excluded by the magnitude (13 c.p.s.) of the coupling constant[8]. This conclusion is already allowed for in part formula (XVI).

The nature of the two carbon bridge between the vinylic CH and CHOH groups of (XVI) was established by the isolation of *meso*-diethylsuccinic acid from oxidation experiments (see below) and supported by the isolation of diethylacrolein from the pyrrolysis of glauconic acid. Part formula (XVI) can therefore be expanded to (XVII). The latter contains 19 carbon atoms and therefore one atom in (XVII), indicated by a C, must, in fact, be allowed for twice over. The final structural detail was settled quickly by the following considerations. Glauconic acid and its derivatives, where appropriate, show one quaternary C-methyl group in their N.M.R. spectra. Formula (XVII) must therefore be modified to (XVIII; R = H) as the unique constitution of glauconic acid. The methylene group in this formula can be identified in the derived glauconic acid ketone (XIX) as an AB quartet ($\tau = 6.677$, 6.96; $J = 15$ c.p.s.). It must, therefore, be placed between two fully substituted carbon atoms as already allowed for in (XIX) and related formulae.

We may now consider the chemistry of glauconic acid in the light of the constitution (XVIII; R = H) now established. It is obvious that this formula does not contain the glauconin carbon skeleton and that the formation of this substance, in fact, gives a misleading clue to the constitutional problem. It seems that glauconic acid must first undergo a COPE[12,13] rearrangement (XVIII; R = H; see arrows) to (XX; R = H) which is then converted to (XXI) by a reversed aldol reaction. The degradation of (XX) to diethylacrolein (I) and

[10] K. BOWDEN, I. M. HEILBRON, E. R. H. JONES, and B. C. L. WEEDON, J. chem. Soc. *1946*, 39.
[11] E. D. BERGMANN, D. GINSBURG, and R. PAPPO, in Org. Reactions *10*, 179 (1959).
[12] A. C. COPE and E. M. HARDY, J. Amer. chem. Soc. *62*, 441 (1940).
[13] A. C. COPE, K. E. HOYLE, and E. M. HARDY, J. Amer. chem. Soc. *63*, 1843 (1941).—A. C. COPE, C. F. HOFMANN, and E. M. HARDY, J. Amer. chem. Soc. *63*, 1852 (1941).

glauconin (VII) then follows by a conventional reversed Michael reaction.

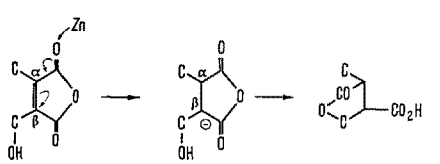


(XXI) (XXII) (XXIII)

(XXIV) (XXV) (XXVI)

The pyrolysis of glauconic acid acetate (XVIII; R = Ac) provides support for these arguments. There is formed an isomeric compound, *isoglauconic acid acetate*, m.p. 257–258°, $[\alpha]_D$ −5°, (c, 1.36), λ_{max} 220 mμ (ε = 10500), ν_{max} 1835, 1776, and 1742 cm^{-1}, which contains one ethylenic linkage, since it furnishes on hydrogenation over 10% palladised charcoal a *dihydroisoglauconic acid acetate*, m.p. 195–202°, $[\alpha]_D$ − 104°, (c, 0.52), $\varepsilon_{220\,m\mu}$ = 300, ν_{max} 1850, 1780, and 1715 cm^{-1}. This derivative is saturated and therefore has two carbocyclic rings. Isoglauconic acid acetate has two vinyl protons (τ = 3.26 and 3.95) each split as a doublet with a coupling constant of about 1 c.p.s. This evidence, supported by that of the ultraviolet absorption spectrum, indicates the presence of an itaconic anhydride residue with the ethylenic linkage terminal (as = CH_2). This was confirmed by the isolation of a good yield of formaldehyde on ozonolysis. If glauconic acid acetate rearranges on pyrolysis to (XX; R = Ac) then a further rearrangement can be envisioned (XX; R = Ac; see arrows) which would give the formula (XXII; R = Ac) for isoglauconic acid acetate. This formula would agree with the fact (N.M.R.) that the compound has no C-methyl groups (apart from those in the two ethyl groups).

The zinc-acetic acid reduction of glauconic acid to dihydroglauconic acid has already been referred to above. This reaction can be understood if one considers that the reaction takes the course shown below.



The donation of electrons from the zinc metal affords an anion (in the β-position) which is stabilised since it is α- to a carbonyl group. A similar reduction of an itaconic anhydride type unit through carbonyl oxygen is not probable since the carbanion formed would not be stabilised in the same way. Isomerisation of the initially formed succinic anhydride by interaction with the allylically placed hydroxyl group would then afford the γ-lactone-acid, dihydroglauconic acid (XXIII). In the case of glauconic acid acetate competition with anionic β-elimination of acetoxyl can be envisaged to give glaucanic acid (XXIV). The N.M.R. spectrum of glaucanic acid is in full accord with this formula.

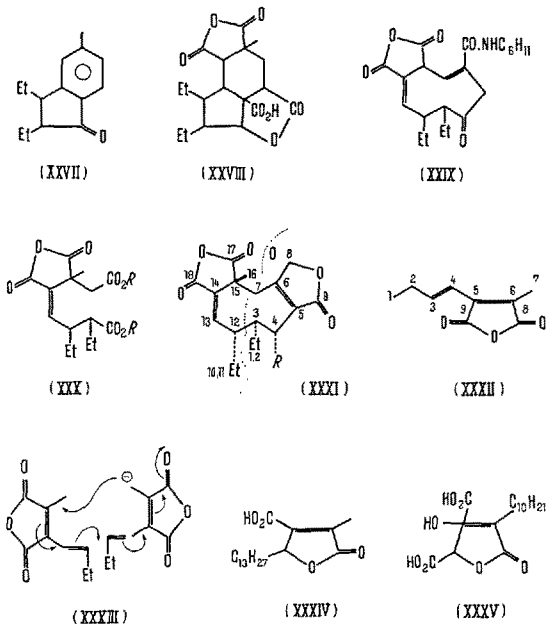
Thepэ‚ uction of glauconic acid ketone with zinc dust and acetic acid has already been discussed at some length above. The saturated bicyclic ketone thereby produced can now be formulated as (XXV), whilst its companion unsaturated ketone can be represented as (XXVI). Further support for formula (XXV) has been secured by dehydrogenation over palladised charcoal which afforded an *aromatic ketone*, $[\alpha]_D \pm 0°$, characterised as an α-hydrindanone by its ultraviolet (λ_{max} 250 mμ (ε = 8100), 274 mμ (ε = 2500), and 294 mμ (ε = 1800)) and infrared (ν_{max} 1700 cm^{-1}) spectra. This compound should have formula (XXVII) and its synthesis is now in progress.

Pyrolysis of dihydroglauconic acid chloride at 200° for 30 min gave an *isodihydroglauconic acid*, $C_{18}H_{22}O_7$, m.p. 227–230°, $[\alpha]_D$ + 5°, (c, 1.00), $\varepsilon_{220\,m\mu}$ = 500, ν_{max} 1860, 1785, and 1730 cm^{-1}, characterised as the *monomethyl ester*, $C_{19}H_{24}O_7$, m.p. 165–167°, $[\alpha]_D$ + 12°, (c, 0.90), $\varepsilon_{220\,m\mu}$ = 800, ν_{max} 1860, 1785, and 1730 cm^{-1}. This compound, which is saturated and therefore bicarbocyclic, is formulated as (XXVIII), being formed by a Michael type reaction between the α-position of the acid chloride and the β-position of the itaconic anhydride type residue.

Reference to several of the reactions of glauconic acid ketone (XIX) has already been made in the text above. This ketone is an extremely sensitive compound which is attacked by many reagents. For example, if the ketone is refluxed with cyclohexylamine carbonate in benzene it affords an *amide*, $C_{23}H_{31}O_5N$, m.p. 178–179°, $[\alpha]_D$ − 26°, (c, 1.06), λ_{max} 218 mμ (ε = 12700), ν_{max} 1830, 1775, 1690, and 1650 cm^{-1}, whose properties are in accord with the constitution (XXIX). The N.M.R. spectrum of this amide shows two vinyl protons, one as a doublet (τ = 3.09; J = 10 c.p.s.) and another as a singlet (τ = 3.44). On the other hand, if glauconic acid ketone (XIX) is reacted under the same conditions with pure cyclohexylamine an *amide-imide*, $C_{27}H_{38}O_5N_2$, m.p. 195–197°, $[\alpha]_D$ − 28°, (c, 0.99), λ_{max} 226 mμ (ε = 15600), ν_{max} 1755, 1700, and 1630 cm^{-1}, is formed. The constitution of this compound is still under investigation.

Oxidation of glauconic acid ketone with chromic acid in acetic acid gave *meso*-diethylsuccinic acid, the identity of which was carefully confirmed. Oxidation of glauconic acid itself under the same conditions afforded the same diethylsuccinic acid and a crystalline *dicarb-*

oxylic acid, $C_{15}H_{20}O_7$, m.p. 202–204°, $[\alpha]_D + 61°$, (c, 1.20), λ_{max} 224 mμ ($\varepsilon = 7450$), ν_{max} 1845, 1750, 1730, and 1690 cm^{-1}. This compound clearly retains the itaconic anhydride type residue. It is formulated as (XXX; R = H). Treatment with diazomethane furnished the *dimethyl ester* (XXX; R = Me), $C_{17}H_{24}O_7$, m.p. 91–92°,



(XXVII) (XXVIII) (XXIX)

(XXX) (XXXI) (XXXII)

(XXXIII) (XXXIV) (XXXV)

$[\alpha]_D + 55°$, (c, 1.30), λ_{max} 223 mμ ($\varepsilon = 9500$), ν_{max} 1830, 1770, and 1730 cm^{-1}. The N.M.R. spectrum of this ester showed the usual vinyl proton doublet at $\tau = 3.28$ ($J = 11$ c.p.s.), the quaternary methyl at $\tau = 8.37$ and methylene absorption (a singlet at $\tau = 6.77$). Further chromic acid oxidation of the diacid (XXX; R = H) gave *meso*-diethylsuccinic acid in trace amounts (paper chromatography).

We now turn to consideration of the stereochemistry of glauconic acid. In all the formulae used so far the vinylic hydrogen of glauconic acid (XVIII; R = H) has been written *cis* with respect to the anhydride residue. This assignment is made[14] because the average position of the vinylic proton in the N.M.R. spectra is at $\tau = 3.10$. The vinylic proton in Δ^2-tetrahydrophthalic anhydride absorbs at $\tau = 2.95$. The best compound for comparison is glaucanic acid (XXIV) whose vinylic proton absorbs at $\tau = 3.00$. In itaconic anhydride the two vinylic protons (one *cis*, one *trans* with respect to the anhydride) differ in absorption by 0.43 p.p.m. The close agreement between the position of absorption of the vinylic proton of glaucanic acid (XXIV) and the model Δ^2-tetrahydrophthalic anhydride provides then good support for the assigned *cis*-configuration.

The relatively large coupling constant for the splitting of the vinylic proton of glauconic acid and its derivatives by the adjacent allylic hydrogen has already been mentioned (see above). This implies[8] that the dihedral angle between the two C-H bonds involved must be about 180°. Examination of molecular models suggests that this condition can only be satisfied if the angular methyl group at C_{15} and the ethyl group at C_{12} are *trans* to each other. The degradation of the molecule to *meso*-diethylsuccinic acid shows that, provided epimerisation has not occurred during the reaction sequence, the two ethyl groups at C_{12} and C_3 must be *cis* to each other in the nine-membered ring. There remains only the configuration of the hydroxyl group for consideration. Perhaps the best evidence on this point comes from the N.M.R. spectrum of isoglauconic acid acetate (XXII; R = Ac). Certain aspects of this spectrum have already been discussed (see above). The proton attached to the carbon bearing the acetoxyl group is split as a doublet ($\tau = 5.09$); with a coupling constant of 8 c.p.s. If the acetoxyl group is indeed attached to a five-membered ring, then the dihedral angle between this C-H bond and the single neighbouring C-H bond can only approximate to 0° or 120°. The magnitude of the coupling constant[8,15] is best explained if this angle be near to 0°, so that the acetoxyl residue and the adjacent ethyl group must be *cis* to each. These tentative conclusions as to the stereochemistry of glauconic acid are summarised (without commitment as to absolute configuration) in the formulae (XXXI; R = OH) for glauconic acid and (XXXI; R = H) for glaucanic acid[16].

It is now opportune to discuss the biogenesis of glauconic and glaucanic acids. In common with many other fungal metabolites we have shown in preliminary experiments[17] that they are derived from acetate[18] (or the equivalent malonate[19]). However, there is an aspect of the constitutions of these compounds which we consider makes them of unusual biogenetic interest. In principle, the constitution of glauconic acid (XXXI; R = OH) may be derived from two units having identical carbon skeletons (XXXII). This we have already implied in the numbering system adopted. The oxidation level of glauconic and glaucanic acids is such that if, in principle, one took the dienoid unit (XXXII), an anion from one molecule could serve as an agent for the construction of the whole carbon skeleton of glaucanic acid with the substituent ethylenic linkages in the correct positions (see (XXXIII)). There are, of

[14] L. M. Jackman and R. H. Wiley, J. chem. Soc. *1960*, 2881.

[15] H. Conroy, in *Advances in Organic Chemistry* (Eds. R. A. Raphael, E. C. Taylor, and H. Wynberg, Interscience Publishers Inc., New York 1960), vol. 2, p. 311.

[16] We have prepared the *m-iodobenzoate* of glauconic acid, $C_{25}H_{23}IO_8$, m.p. 197–198°, $[\alpha]_D + 89°$ (c, 0.64), ν_{max} 1840, 1770, and 1725 cm^{-1}. This substance is currently under examination by Professor J. M. Robertson, Dr. G. A. Sim et al., in order to define with certainty the stereochemistry of glauconic acid.

[17] Carried out in collaboration with Mr. L. D. S. Godinho.

[18] A. J. Birch, *Fortschritte der Chemie organischer Naturstoffe* (Springer Verlag, 1957), vol. XIV, p. 187, and references there cited.

[19] R. Bentley and J. G. Keil, Proc. chem. Soc. *1961*, 111.—J. D. Bu'Lock and H. M. Smalley, Proc. chem. Soc. *1961*, 209.

course, many equivalent representations. If this hypothesis be granted then the hydroxyl group of glauconic acid would be introduced at a later stage in the biogenesis, possibly by biochemical hydroxylation of glaucanic acid.

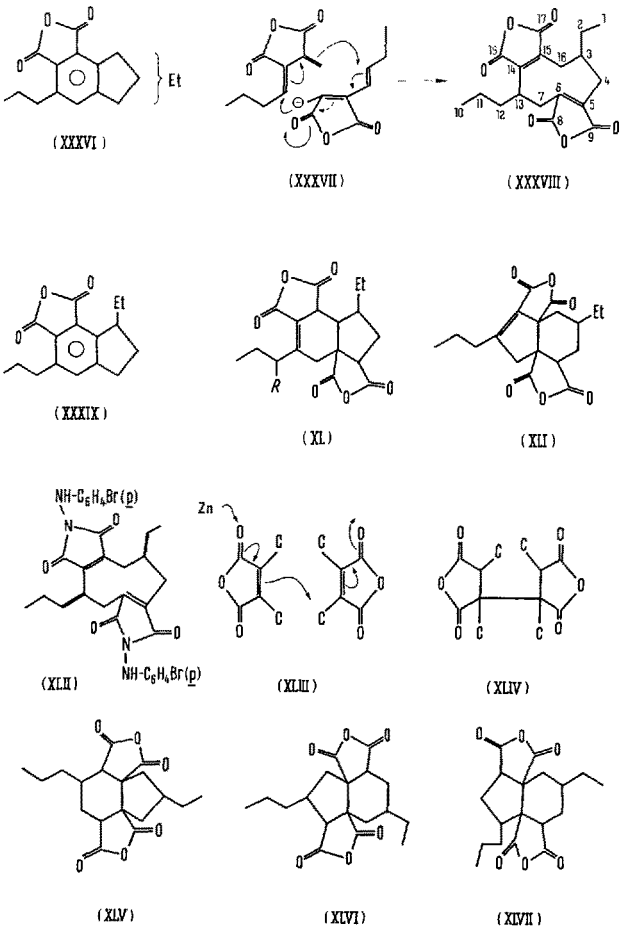
The carbon skeleton of (XXXII) is readily derivable from an intermediate citric acid and it is possible that acids of this kind are intermediates in the biogenesis of such compounds as lichesterenic acid (XXXIV)[20], mineoluteic acid (XXXV)[21] and many other analogous fungal products. We are at present engaged on a study of the biogenesis of glauconic acid from C_2 and higher units to test the hypotheses here enunciated.

The literature describes the isolation and characterisation of an isomer of glaucanic acid, byssochlamic acid, which also contains two anhydride rings[22]. Byssochlamic acid is obtained from *Byssochlamys fulva* Olliver and Smith. From the outset we suspected that glaucanic and byssochlamic acids were related biogenetically. Byssochlamic acid showed $\lambda_{max}^{cyclohexane}$ 244 mμ ($\varepsilon = 9100$), ν_{max} 1845 and 1770 cm^{-1} and showed no vinyl protons in its N.M.R. spectrum. This evidence indicated to us that the molecule contained two maleic anhydride residues and must, therefore, be monocarbocyclic. On Kuhn-Roth oxidation byssochlamic acid gave two moles of volatile acid. The N.M.R. spectrum showed that neither of the C-methyl groups which must, therefore, be present was quaternary and made it probable that two ethyl or higher alkyl groups were contained in the molecule. Now it had been demonstrated in unpublished work by COOK, LOUDON, and PATON[23] that on heating to 200° byssochlamic acid was converted into an isomer, *isobyssochlamic acid*, $C_{18}H_{20}O_6$, m.p. 153°, $[\alpha]_D$ + 27° (c, 1.00), $\lambda_{max}^{cyclohexane}$ 236 mμ ($\varepsilon = 6300$), ν_{max} 1855, 1830, 1780, and 1770 cm^{-1}, characterised (by dissolution in alkali and acidification) as the *hydrate*, a *dicarboxylic acid anhydride*, $C_{18}H_{22}O_7$, m.p. 173–183° (decomp.), $[\alpha]_D$ + 24° (c, 1.30), λ_{max} 234 mμ ($\varepsilon = 3300$), ν_{max} 1840, 1780, 1700, and 1690 cm^{-1}. At the m.p. this hydrate gave back isobyssochlamic acid. Hydrogenation of isobyssochlamic acid in acetic acid over 10% palladised charcoal gives[23] the saturated *dihydroisobyssochlamic acid*, $C_{18}H_{22}O_6$, m.p. 130°, $[\alpha]_D$ – 16° (c, 0.89), $\varepsilon_{220\,m\mu} = 245$, ν_{max} 1840 and 1775 cm^{-1}, which also gives a stable *hydrate*, $C_{18}H_{24}O_7$, m.p. 190–200° (decomp.). From this evidence it is clear that the conversion of byssochlamic to isobyssochlamic acid is a cyclisation of a mono- to a bi-carbocyclic system. Furthermore, dehydrogenation of byssochlamic acid over platinum at 300° affords[24] a *compound*, $C_{16}H_{18}O_3$, m.p. 91°, $[\alpha]_D \pm 0°$. We have shown that this compound has $\lambda_{max}^{cyclohexane}$ 224 and 232 mμ ($\varepsilon = 26000$ and 18000 respectively), ν_{max} 1835 and 1770 cm^{-1}, and that it contains two C-methyl groups by Kuhn-Roth determination. The N.M.R. spectrum showed that both these methyl groups were present as ethyl or higher alkyl groups and revealed the presence of one aromatic proton at $\tau = 2.72$. These facts establish that the compound, $C_{16}H_{18}O_3$, must be a substituted phthalic anhydride, the position of the aromatic proton in the N.M.R. spectrum suggesting that it is separated from the anhydride residue by one alkyl group. Dehydrogenation of isobyssochlamic acid gave the same substituted phthalic anhydride in improved yield. Assuming that no extra rearrangement is involved in this dehydrogenation, then isobyssochlamic acid must contain a six-membered ring bearing an itaconic anhydride type residue. A second carbocyclic ring must be present and, since this occupies two positions in *ortho*-relationship on the benzene ring of the $C_{16}H_{18}O_3$ compound, we thought that it was probably a five-membered ring. The isolation of benzene-pentacarboxylic acid from nitric acid oxidation[25] of the $C_{16}H_{18}O_3$ confirms that five substituents are attached to this benzene ring. There must be one alkyl group attached to the benzene ring of the $C_{16}H_{18}O_3$ compound (see above) and since this furnishes one mole of volatile acid on oxidation it must be at least an *n*-propyl group. Assuming that any tetralin-type compound would have dehydrogenated further, we concluded that the $C_{16}H_{18}O_3$ compound was a hydrindane with an anhydride residue and an *n*-propyl group attached to the benzene ring. By exclusion there must be one ethyl group attached to the cyclopentane ring and, therefore, all the properties of the $C_{16}H_{18}O_3$ compound can be summarised in the expression (XXXVI). The mode of formation of isobyssochlamic acid and its relationship to (XXXVI) provides good evidence that byssochlamic acid itself contains a nine-membered ring.

If one now makes the hypothesis that byssochlamic acid is formed from the same biogenetic units, (XXXII) or equivalent, by the same type of mechanism, (XXXIII) or equivalent, as operates in the biogenesis of glaucanic acid, then one can (as first pointed out by one of us (J.K.S.)) advance a plausible formula. Attaching the units to each other as in scheme (XXXVII) (or equivalent operation) affords (XXXVIII) as the constitution of byssochlamic acid. The $C_{16}H_{18}O_3$ compound, if formed without complication, would then be (XXXIX) and isobyssobhlamic acid, which has no vinyl hydrogen in its N.M.R. spectrum and whose ultraviolet spectrum indicates a fully substituted itaconic anhydride type residue, would be (XL; *R*=H), or equivalent formulation. Bromination of isobyssochlamic

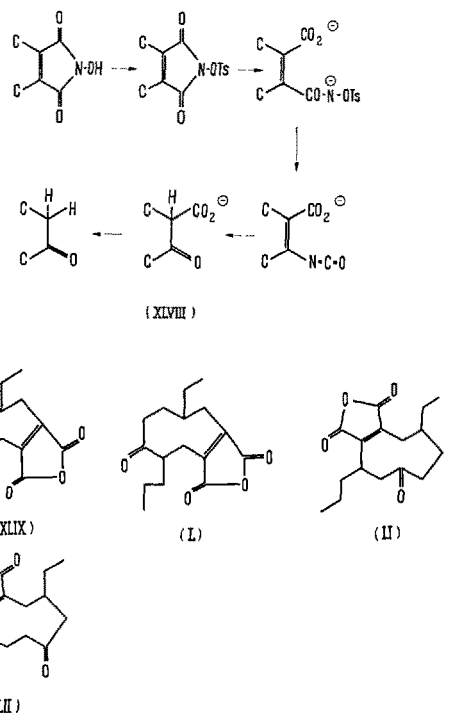
[20] M. ASANO and T. KANEMATSU, Ber. dtsch. chem. Ges. *65*, 1175 (1932).
[21] J. H. BIRKINSHAW and H. RAISTRICK, Biochem. J. *28*, 828 (1934).
[22] H. RAISTRICK and G. SMITH, Biochem. J. *27*, 1814 (1933).
[23] J. W. COOK, J. D. LOUDON, and R. P. PATON; see R. P. PATON, Ph. D. Thesis, Glasgow (1954).
[24] J. N. ASHLEY, P. CLUTTERBUCK, and H. RAISTRICK, unpublished observations: see R. P. PATON, Ph. D. Thesis, Glasgow (1954).
[25] Unpublished experiments by Dr. J. W. COOK, F.R.S.

acid with N-bromosuccinimide gave a *monobromo-derivative*, m.p. 180–185°, $[\alpha]_D$ – 31° (c, 1.00), $\lambda_{max}^{cyclohexane}$ 240 mμ ($\varepsilon = 8600$), ν_{max} 1830 and 1780 cm^{-1}. The N.M.R. spectrum of this bromo-compound showed a triplet at $\tau = 4.14$ indicative of one hydrogen attached to carbon bearing bromine, the carbon atom being flanked by a methylene group. The part structure ($-CH_2-CHBr$) can be accommodated by the expression (XL; R = Br).



The major difficulty in accepting formula (XL) for isobyssochlamic acid is that its relationship to byssochlamic acid is not, from the mechanistic point of view, very clear. Alternative formulae such as (XLI) could explain all the facts about isobyssochlamic acid except that its dehydrogenation to (XXXIX) would involve a further rearrangement. Because of these difficulties we had not reached a firm conclusion as to the structure of byssochlamic acid at a time when Professor J. M. Robertson, F.R.S., Dr. G. A. Sim et al.[26] had been able to complete an X-ray determination of the structure of byssochlamic acid bis-*p*-bromophenylhydrazide. This compound has the constitution and configuration, apart from absolute configuration, depicted in (XLII) and thus proves the correctness of formula (XXXVIII). The *bis-P-bromophenylhydrazide*, m.p. 164–166°, $[\alpha]_D$ – 97° (c, 0.30), λ_{max} 236 mμ ($\varepsilon = 41000$), 292 mμ ($\varepsilon = 3100$), ν_{max} 1780 and 1728 cm^{-1}, was prepared by treating byssochlamic acid with *p*-bromophenylhydrazine in chloroform solution.

Further aspects of the chemistry of byssochlamic acid can now be rationalised in terms of formula (XXXVIII). As with glauconic acid, reduction of byssochlamic acid with zinc dust and acetic acid gave interesting results. There was formed, after the appropriate working up procedure, a saturated *'dihydrobyssochlamic acid'*, $C_{18}H_{22}O_6$, m.p. 120°, $[\alpha]_D$ – 54° (c, 1.00), $\varepsilon_{220\,m\mu} = 350$, ν_{max} 1840 and 1775 cm^{-1}, further characterised as the *hydrate*[21], $C_{18}H_{24}O_7$, m.p. 241°, $[\alpha]_D$ + 15° (c, 0.90 in acetone), $\varepsilon_{220\,m\mu} = 450$, ν_{max} 1830, 1780, and 1700 cm^{-1}, which was reconverted into its progenitor on warming with acetyl chloride. We consider that reduction of one of the anhydride systems of byssochlamic acid gives an anion which adds to the second anhydride system as illustrated in (XLIII) → → (XLIV). Accepting only formulae with fused five- and six-membered rings three formulae ((XLV), (XLVI) and (XLVII)) can be written for 'dihydrobyssochlamic acid'. We are unable to distinguish between them at the present time.



The ethylenic linkages of byssochlamic acid are, not surprisingly, very resistant to attack by electrophilic reagents. We decided, therefore, to attempt to modify the maleic anhydride systems by a novel Hofmann degradation. One of the anhydride rings was converted to N-hydroxy-imide by reaction with hydroxylamine.

[26] T. A. Hamor, I. C. Paul, J. Monteath Robertson, and G. A. Sim, Exper. *18*, 352 (1962).

The N-hydroxy-imide was treated with toluene-*p*-sulphonyl chloride and then with 1*N* aqueous sodium hydroxide at 95°. This gave two *ketones*, $C_{16}H_{22}O_4$, (A) m.p. 110°, $[\alpha]_D$ – 79° (c, 0.90), $\lambda_{max}^{cyclohexane}$ 252 mμ ($\varepsilon = 4600$), ν_{max} 1860, 1760, and 1695 cm^{-1}, and (B) m.p. 155°, $[\alpha]_D$ + 6° (c, 0.95), $\lambda_{max}^{cyclohexane}$ 255 mμ ($\varepsilon = 5500$), ν_{max} 1845, 1760, and 1686 cm^{-1}. These compounds must be formed according to the sequence (XLVIII) and therefore have two out of the four formulae ((XLIX), (L), (LI), and (LII)). We are currently degrading these ketones further to establish their constitutions and to obtain evidence as to the absolute configuration of byssochlamic acid[27,28].

Zusammenfassung. Die Struktur der Pilzstoffwechselprodukte Glaucon- und Glaucansäure ($C_{18}H_{20}O_7$ bzw. $C_{18}H_{20}O_6$) aus *Penicill. purp.* wird in der Hauptsache durch das Studium der Reduktionsprodukte ermittelt. Diese Verbindungen enthalten zwei fünfgliedrige Säureanhydrid-Gruppierungen, die mit einem eigenartigen doppelt ungesättigten neungliedrigen Ringsystem gekuppelt sind, das unter verschiedenen Bedingungen in das Indangerüst übergeführt werden kann.

Wie schon früher beschrieben, wird Glauconsäure leicht pyrolytisch zu Diäthylacrolein und Glauconin abgebaut. Die Formel des Glauconins, die durch Spektralmessungen und Synthese bewiesen wurde, ist für die Konstitutionsaufklärung der Glauconsäure in gewisser Hinsicht irreführend, da das Kohlenstoffskelett des Glauconins nicht schon in der Glauconsäure vorliegt, sondern erst durch eine Umlagerung unter Bildung neuer C–C-Bindungen bei der Spaltung der Glauconsäure entsteht.

Die Biogenese der beiden Säuren wird diskutiert; sie scheint auf der Verknüpfung zweier Fragmente mit neun Kohlenstoffatomen und identischem Gerüst zu beruhen. Die Annahme einer andersartigen Verknüpfung der beiden Fragmente führt zu einer möglichen Struktur für Byssochlamsäure, $C_{18}H_{20}O_7$. Die so abgeleitete Konstitution wird durch röntgenkristallographische Strukturbestimmung bestätigt.

Byssochlamsäure, aus *Byssochlamys fulva*, enthält ebenfalls ein neungliedriges, doppelt ungesättigtes Ringsystem sowie zwei fünfgliedrige Säureanhydrid-Gruppierungen und zeigt die Tendenz, Indanderivate zu bilden. Ihr chemisches Verhalten wird anhand der durch Röntgenstrahlenkristallographie bestimmten Struktur diskutiert.

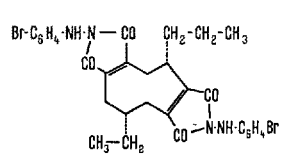
[27] Unless stated otherwise $[\alpha]_D$ were taken in $CHCl_3$, ultraviolet absorption spectra in ethanol and infrared spectra as Nujol mulls. All m.p.s. were determined on the Kofler block. N.M.R. spectra were determined in $CDCl_3$ and τ values given refer to the centre of multiple absorption where present. We thank Dr. J. W. LOWN and Mr. R. G. FOSTER for some of these determinations.

[28] *Acknowledgments.* We express our deep appreciation to Professor H. RAISTRICK, F.R.S., for his constant encouragement and for supplies of glauconic and byssochlamic acids. We thank Professor J. H. BIRKINSHAW, Mr. G. SMITH, and Dr. C. E. STICKINGS for very helpful advice on the culture of *Penicillium purpurogenum* and for facilities generously placed at our disposal. Dr. J. W. COOK, F.R.S., and Dr. J. D. LOUDON kindly permitted us to see, and to quote from, their unpublished experiments. Dr. E. R. S. WINTER of Messrs. J. and E. Sturge (Birmingham) is thanked for the supply of glauconic acid at critical moments. Dr. R. H. B. GALT, Dr. H. HOFMANN, Dr. H. H. A. LINDE, and Dr. J. C. E. PECHER made constructive contributions to attempted syntheses of glauconin and we thank them cordially. Dr. R. I. REED (Glasgow) kindly determined the M.W.s of glauconic acid acetate and of several other compounds described in this communication.

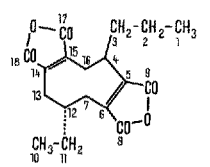
The Structure of Byssochlamic Acid

By T. A. HAMOR, I. C. PAUL, J. MONTEATH ROBERTSON, and G. A. SIM*

Byssochlamic acid, the characteristic metabolite of *Byssochlamys fulva*, was first isolated in 1933 by RAISTRICK and SMITH[1]. In the course of subsequent studies of the related byssochlamic, glauconic, and glaucanic acids BALDWIN, BARTON, BLOOMER, JACKMAN, RODRIGUEZ-HAHN, and SUTHERLAND[2] prepared a beautifully crystalline derivative of byssochlamic acid by reacting it with *p*-bromophenylhydrazine. We have elucidated the crystal structure of this derivative by detailed X-ray analysis and since our results define the constitution and relative stereochemistry of the derivative to be as in (I) it follows that the structure of byssochlamic acid is represented by (II). At the outset of the X-ray study the only chemical information available to us concerning byssochlamic acid was that it contains two anhydride rings and has molecular formula $C_{18}H_{20}O_6$[1,2].



(I)



(II)

* Chemistry Department, The University, Glasgow (Scotland).

[1] H. RAISTRICK and G. SMITH, Biochem. J. *27*, 1814 (1933).

[2] J. E. BALDWIN, D. H. R. BARTON, J. L. BLOOMER, L. M. JACKMAN, Miss L. RODRIGUEZ-HAHN, and J. K. SUTHERLAND, Experientia *18*, 345 (1962).