1027. *Aucubin.*

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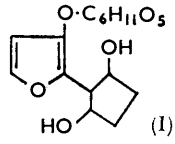
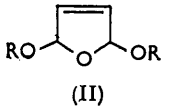
Reduction of aucubin by lithium and ammonia gave a dideoxyaucubin (isolated as its tetra-acetate). The corresponding aglucone proved stable and its reactions confirm structure (V) proposed for aucubin by Japanese workers.[1] A preliminary account [2] of our work has been published. Swiss [3,4] workers have also published supporting evidence for structure (V).

The glucoside of *Aucuba japonica*, Thunb., was first obtained pure by Bourquelot and Hérissey [5] who named it aucubin (aucuboside). Later workers [6] showed aucubin to be identical with rhinanthin isolated [7] in 1870 from the seeds of *Rhinanthus* species, but on the recommendation of Bridel and Braecke [6] the name aucubin has been retained for this glucoside as the original rhinanthin was not pure. Aucubin is of very wide occurrence in Nature [8] and is found in large quantities in individual plants. Seeds of *Melampyrum* [6] and of *Rhinanthus* species [7] have been known since classical times as the troublesome contaminants of wheat which cause the resulting bread to be black. This blackening is due to aucubin. Aucubin appears to be the active principle [9] of *Plantago* species, for many years recommended in the French Pharmacopœia as a general panacea. It increases the rate of removal of uric acid from the body,[10] and the aglucone has antibiotic activity.[11]

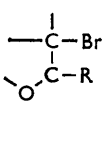
Bourquelot and Hérissey [5] showed aucubin to be a β-glucoside whose aglucone is very unstable and rapidly affords an insoluble black amorphous powder on treatment with acid. Little progress was made with structural investigation until Karrer and Schmid [12] prepared derivatives of the aglucone. They showed that aucubin has the molecular formula $C_{15}H_{22}O_9$, containing six hydroxyl groups, four of which are accounted for by the glucose moiety, and two double bonds and an inert oxygen atom. The remaining two oxygen atoms are accounted for by the glucopyranose ring and the glycosidic link. Catalytic hydrogenation of aucubin hexa-acetate gave a saturated tetrahydro-derivative. Acid treatment of tetrahydroaucubin afforded anhydrotetrahydroaucubigenin formulated as (IV) with one secondary hydroxyl group and two inert oxygen atoms. Karrer and



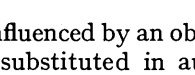
(I)

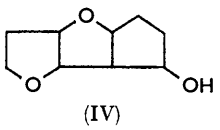


(II)



(III) a : R = OAlK

b : R = 



(IV)

Schmid's structural proposal (I) for aucubin was influenced by an observation by Bergmann and Michalis [13] that one atom of bromine is substituted in aucubin hexa-acetate in methanol and by their own observation that lead tetra-acetate introduces a further acetoxy-group into aucubin hexa-acetate. Karrer and Schmid interpreted these reactions as indicating the presence in aucubin of a furan ring, but this is not in accord with the subsequent work of Clauson-Kaas and his collaborators on the reaction between furan

[1] Fujise, Obara, and Uda, *Chem. and Ind.*, 1960, 289; Fujise, Uda, Ishikawa, Obara, and Fujino, *ibid.*, 1959, 954.
[2] Grimshaw and Juneja, *Chem. and Ind.*, 1960, 656.
[3] Wendt, Haegele, Simonitsch, and Schmid, *Helv. Chim. Acta*, 1960, **43**, 1440.
[4] Haegele, Kaplan, and Schmid, *Tetrahedron Letters*, 1961, No. 3, 110.
[5] Bourquelot and Hérissey, *Compt. rend.*, 1902, **134**, 1441; 1904, **138**, 1114; *Ann. Chim. Phys.*, 1905, **4**, 289.
[6] Bridel and Braecke, *Compt. rend.*, 1922, **175**, 533; 640.
[7] Ludwig, *Arch. Pharm.*, 1870, **192**, 199; 1872, **199**, 6.
[8] Paris and Chaslot, *Ann. Pharm. franç.*, 1955, **13**, 648.
[9] Bourdier, *J. Pharm. Chim.*, 1907, **26**, 254.
[10] Kato, *Folia Pharmacol. Japonica*, 1946, **42**, 37 (*Chem. Abs.*, 1953, **47**, 1843).
[11] Rombouts and Links, *Experientia*, 1956, **12**, 78.
[12] Karrer and Schmid, *Helv. Chim. Acta*, 1946, **29**, 525.
[13] Bergmann and Michalis, *Ber.*, 1927, **60**, 935.

and bromine in methanol [14] to give the dihydro-diether (II; R = Me) and the action of lead tetra-acetate on furan [15] to give the corresponding diacetate (II; R = Ac). During the course of our work Japanese authors [1] proposed structure (V) for aucubin and confirmed the nature of the carbon skeleton by converting anhydrotetrahydroaucubigenin, now formulated as (VI), into the acid (VII) whose anhydride methyl ester showed an infrared spectrum identical with that of the synthetic racemate. Our work, briefly reported elsewhere,[2] provides alternative evidence for the nature and arrangement of the functional groups. Schmid and his collaborators [3,4] also published alternative evidence confirming this structure for aucubin and from nulcear magnetic resonance evidence [4] favour a *cis*-junction of the rings.

We first examined Bergmann and Michalis's " bromoaucubin hexa-acetate," m. p. 182°. This may be prepared from aucubin hexa-acetate by the action of bromine in methanol [13] or by using *N*-bromosuccinimide and crystallising the product from methanol.[12] Analysis revealed a methoxyl group (missed by previous workers). Methanol which was liberated by acid hydrolysis of this derivative was characterised as formaldehyde dimethone. A different bromo-compound was obtained on bromination of aucubin hexa-acetate in ethanol. Thus bromination in methanol results in the addition of the elements of methyl hypobromite across one double bond. This observation led us to suppose that aucubin contains an enol-ether ethylenic link and to seek an explanation of the " aromatic substitution " of aucubin on this basis.

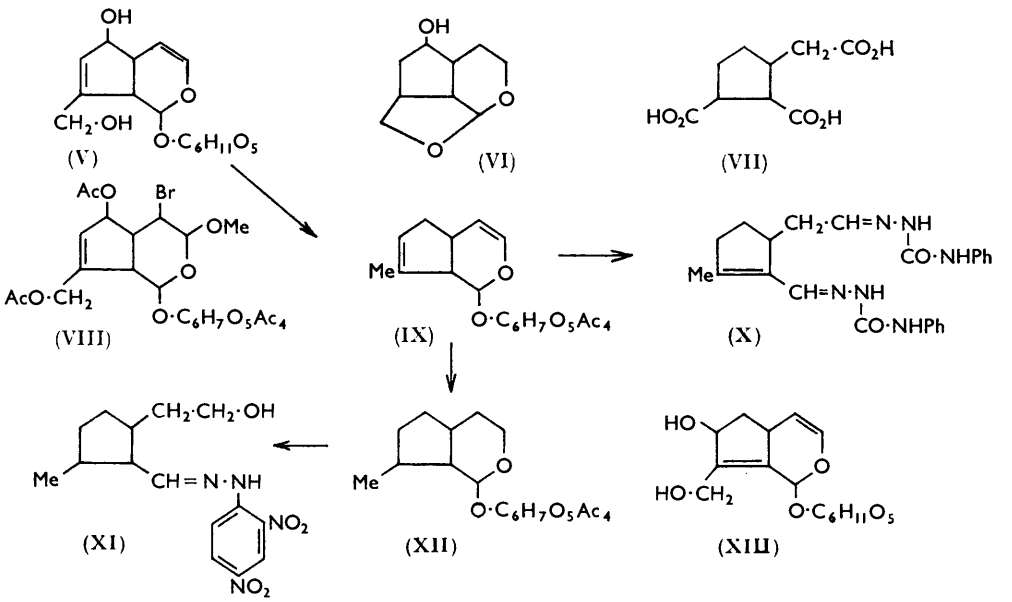
It is well known that enol ethers rapidly form addition products with two atoms of chlorine or bromine in an indifferent solvent and that the resulting α-halogeno-ether is easily converted into an α-alkoxy-ether by reaction with an alcohol.[16] Thus it seems readily acceptable that in alcoholic solution the final product of bromination of an enol-ether will be the ether bromide (IIIa), the aucubin derivative being (VIII). Such a reaction is shown by the glucoside plumieride [17] which also contains an enol-ether grouping. The enol-ether double bond also combines with *N*-bromosuccinimide to give the addition product (IIIb) which in refluxing alcohol affords [18] the ether bromide (IIIa) in an analogous manner to Karrer and Schmid's bromination procedure for aucubin hexa-acetate. Dihydropyran with lead tetra-acetate gives a substitution product,[19] as does aucubin hexa-acetate. With 2,3-dihydrofuran, however, this reagent reacts in an alternative manner to give 2,3-diacetoxytetrahydrofuran.[20] Thus one double bond and the inert oxygen atom of the aucubin aglucone can be satisfactorily placed in an enol-ether system.

Part of the difficulty in the investigation of aucubin has been associated with the instability of the aglucone and this in turn must be due to the highly unsaturated carbonyl systems produced on hydrolysis. Evidence from the hydrogenation experiments mentioned above suggested that one hydroxyl group at least is allylic and accordingly the substance was treated with lithium and ethanol in ammonia in an attempt to remove some of the functional groups. The product, $C_{15}H_{22}O_7$, of this reaction is a dideoxyaucubin, isolated as its tetra-acetyl derivative, $C_{15}H_{18}O_7Ac_4$ (IX). Alkaline hydrolysis followed by acid treatment gave glucose and a comparatively stable, steam-volatile, water-soluble aglucone, dideoxyaucubigenin. Tetra-*O*-acetyldideoxyaucubin formed a methyl ether bromide and absorbed two mol. of hydrogen on catalytic hydrogenation; it therefore contains two double bonds, one in the intact enol-ether grouping. Two different tetrahydro-derivatives were obtained when hydrogenation was carried out in mildly acid solution, but only one of them was obtained in neutral solution; they are presumably stereoisomers (XII).

[14] Clauson-Kaas, Limborg, and Fakstorp, *Acta Chem. Scand.*, 1948, **2**, 109.
[15] Clauson-Kaas and Elming, *Acta Chem. Scand.*, 1952, **6**, 535; Elming, *ibid.*, p. 578.
[16] Wislicenus, *Annalen*, 1878, **192**, 111; Riobé, *Bull. Soc. chim. France*, 1951, 829.
[17] Halpern and Schmid, *Helv. Chim. Acta*, 1958, **41**, 1109.
[18] Paul and Tchelitcheff, *Compt. rend.*, 1953, **236**, 1968.
[19] Hurd and Edwards, *J. Org. Chem.*, 1954, **19**, 1319.
[20] Normant, *Compt. rend.*, 1949, **228**, 102.

The dideoxyaucubigenin was characterised as the bisphenylsemicarbazone (X) of an optically active dicarbonyl compound, $C_9H_{12}O_2$. Kuhn–Roth oxidation of this derivative indicated the presence of one *C*-Me group, in contrast to aucubin which contains none. The ultraviolet absorption of the phenylsemicarbazone, $\lambda_{max.}$ 248 and 286 mμ, corresponds to the presence of derivatives of an isolated and an αβ-unsaturated carbonyl group.[21] Acid hydrolysis of tetra-*O*-acetyldideoxytetrahydroaucubin, m. p. 91°, gave a steam-volatile aglucone characterised as the 2,4-dinitrophenylhydrazone (XI) of a monocarbonyl compound, $C_9H_{16}O_2$.

These results are reconcilable with the presence in aucubin of (*a*) an allylic [22] primary hydroxyl group which gives rise to the new *C*-Me group and which must also be involved in forming the second ethereal oxygen group of anhydrotetrahydroaucubigenin (VI); (*b*) a further allylic hydroxyl group which must become the secondary hydroxyl function of anhydrotetrahydroaucubigenin where it is present in a five-membered ring since the corresponding ketone shows $\nu_{max.}$ 1747 cm.$^{-1}$; (*c*) the system C=C·O·C·O·$C_6H_{11}O_5$ as part of a ring. The last system on hydrolysis gives rise to the two carbonyl groups detectable in dideoxyaucubigenin and the one carbonyl group detectable in the hydrogenated compound



the latter group arising from the acetal system. The *C*-Me group of dideoxyaucubin is unlikely to be attached to the system (*c*) since on the one hand the corresponding aglucone does not give the iodoform reaction and on the other hexa-*O*-acetylaucubin methyl ether bromide is inert [13] towards nucleophilic substitution. A band in the infrared spectrum of aucubin at 1655 cm.$^{-1}$ can be attributed to the enol-ether group.[23] It is present in the spectra of hexa-*O*-acetylaucubin and tetra-*O*-acetyldideoxyaucubin, but is absent from the spectra of the bromo-methyl ethers and tetrahydro-derivatives. Neither aucubin nor tetra-*O*-acetyldideoxyaucubin shows selective ultraviolet absorption corresponding to conjugated double bonds: the system C=C·C=C·O in particular must be absent. The αβ-unsaturated carbonyl chromophore in the dideoxyaucubigenin derivative must be produced therefore by conjugation of one double bond with the carbonyl group formed by hydrolysis of the acetal. This acetal-oxygen is unlikely to be in an allyl ether position

[21] Grammaticakis, *Bull. Soc. chim. France*, 1949, 410.
[22] Birch, *Quart. Rev.*, 1950, **4**, 71.
[23] Davison and Bates, *J.*, 1953, 2607; Meakins, *J.*, 1953, 4170.

since hydrogenolysis should then have occurred with ease during reduction with lithium in ammonia.

All this evidence can be accommodated by the formula (V) proposed for aucubin by the Japanese workers. In particular, chemical evidence is presented for the unusual type of heterocyclic ring. We had deduced formula (V) as being the most likely for aucubin as the ease with which anhydrotetrahydroaucubigenin (VI) is formed in acid solution severely restricts structural possibilities. We rejected structure (XIII) suggested by Schmid,[3] though later withdrawn,[4] since this formula contains an allylic acetal group.

Experimental

M. p.s were taken on a Kofler block standardised against substances of known m. p. Light petroleum of b. p. 60—80° was used. Ultraviolet spectra were measured for absolute ethanol solutions.

Isolation of Aucubin.—Trim and Hill's procedure [24] was used. *Aucuba japonica* leaves collected from shrubs growing in Heaton Mersey, Stockport, at intervals from June to October afforded 1—2% of their fresh weight as aucubin. Aucubin hydrate crystallised from 90% alcohol as colourless needles, m. p. 180—181°, showing no selective absorption in the ultraviolet region at wavelengths longer than 215 mμ, and a band in the infrared region at 1655 cm.$^{-1}$ (Nujol mull). Aucubin hexa-acetate, needles, m. p. 128° (from aqueous methanol), showed infrared bands at 1755 and 1655 cm.$^{-1}$ (in CCl_4).

Hexa-O-acetyl-4-bromo-3,4-dihydro-3-methoxyaucubin (VIII).—Prepared according to the procedure of Bergmann and Michalis,[13] this *compound* crystallised from ethyl acetate–light petroleum as colourless needles, m. p. 181—182° (lit.,[13] m. p. 182°) (Found: Br, 11·6; OMe, 4·6. $C_{23}H_{37}BrO_{16}$ requires Br, 11·3; OMe, 4·4%), $\nu_{max.}$ 1747 cm.$^{-1}$ (in Nujol mull). A sample (0·3 g.) was refluxed with N-sulphuric acid (25 ml.) for 30 min., then about half of the solvent was distilled off. This distillate was redistilled from sodium carbonate and the second distillate again distilled from a little sodium dichromate and sulphuric acid. The final distillate gave formaldehyde dimethone, m. p. and mixed m. p. 189°.

Hexa-*O*-acetylaucubin (0·2 g.), dissolved in ethanol (20 ml.), was cooled to 0° and bromine (0·057 g.) in ethanol (6 ml.) slowly added. After 2 hr., water was added and the precipitate collected. The analogous *ethyl ether* crystallised from ethyl acetate–light petroleum as needles, m. p. 117—118° (with resolidification and m. p. 159—160°) (Found: C, 48·0; H, 5·4; Br, 11·25. $C_{29}H_{39}BrO_{16}$ requires C, 48·1; H, 5·4; Br, 11·1%).

Anhydrotetrahydroaucubigenin (VI). Aucubin was hydrogenated and the products were treated with dilute hydrochloric acid as described by Karrer and Schmid.[12] The product was isolated by continuous ether extraction followed by chromatography of the extract, in chloroform, over alkaline alumina (Peter Spence's grade H). Elution with chloroform afforded, after removal of the solvent, anhydrotetrahydroaucubigenin, needles (from ether), m. p. 89—90° (lit.,[12] m. p. 90·5°), in yields similar to those previously reported. The corresponding ketone showed an infrared band at 1747 cm.$^{-1}$ (in CCl_4).

Dideoxyaucubin Tetra-acetate (IX).—Lithium (1 g.) was added in 2 hr. with stirring to aucubin hydrate (1 g.) in liquid ammonia (200 ml.) and ethanol (6 ml.). After an excess of lithium had remained for 1 hr. this was destroyed by addition of ammonium sulphate. Ethanol and water were then added and the ammonia allowed to evaporate, then the solution was saturated with carbon dioxide and evaporated to dryness. The residue was extracted with hot methanol. Evaporation of the methanol solution left a gum which was left overnight in pyridine (6 ml.) and acetic anhydride (4 ml.). Addition of water precipitated crystals which after several recrystallisations from methanol afforded *dideoxyaucubin tetra-acetate* as needles (0·45 g.), m. p. 137—138°, $[\alpha]_D^{22}$ −142° (*c* 0·5 in $CHCl_3$) (Found: C, 57·3; H, 6·3; Ac, 31·3. $C_{23}H_{30}O_{11}$ requires C, 57·4; H, 6·2; 4Ac, 35·8%), slowly becoming yellow in air. The compound showed no selective ultraviolet absorption at $\lambda > 215$ mμ, and infrared bands at 1760, 1744, and 1665 cm.$^{-1}$ (in Nujol mull). The free glucoside was a gum.

Bromination of Dideoxyaucubin Tetra-acetate.—The above acetate (0·1 g.), dissolved in methanol (10 ml.), was cooled to 0° and bromine (0·034 g.) in methanol (3·4 ml.) slowly added. Addition of water precipitated the *bromo-methoxy-adduct* which crystallised from light petroleum

[24] Trim and Hill, *Biochem. J.*, 1952, **50**, 310.

as needles, m. p. 124—126° (decomp.) (Found: C, 48·8; H, 5·8; Br, 14·0; OMe, 5·7. $C_{24}H_{33}BrO_{12}$ requires C, 48·6; H, 5·6; Br, 13·5; OMe, 5·2%).

Tetrahydrodideoxyaucubin Tetra-acetate (XII).—Dideoxyaucubin tetra-acetate (0·15 g.) in ethyl acetate containing a trace of acetic acid was hydrogenated over Adams catalyst (30 mg.) at room temperature and pressure (uptake 21·4 ml. at N.T.P.; $2H_2 = 20·0$ ml.; 20 min.). Catalyst and solvent were removed and the residue fractionally crystallised from aqueous methanol to give a less soluble *tetrahydro-derivative* as laths (0·02 g.), m. p. 141—142°, $[\alpha]_D^{23}$ +240° (*c* 0·3 in MeOH) (Found: C, 56·7; H, 7·1. $C_{23}H_{34}O_{11}$ requires C, 56·7; H, 7·0%), and a more soluble *tetrahydro-derivative* as square plates (0·08 g.), m. p. 90—91°, $[\alpha]_D^{23}$ +97° (*c* 0·5 in MeOH) (Found: C, 57·0; H, 6·95%). When hydrogenation was carried out with rigorous exclusion of acid then only the derivative of m. p. 91° was obtained.

Dideoxyaucubigenin Bisphenylsemicarbazone (X).—Dideoxyaucubigenin tetra-acetate (0·17 g.) was refluxed for 30 min. under nitrogen with N-sodium hydroxide (10 ml.), then 2N-sulphuric acid (10 ml.) was added. After 1 hour's refluxing under nitrogen the clear solution was steam-distilled until the distillate no longer gave an instantaneous precipitate with Brady's reagent. This distillate, $\lambda_{max.}$ 259 mμ, did not give the iodoform test and afforded the *bisphenylsemicarbazone*, needles (from ethanol), m. p. 203—204° (decomp.), $[\alpha]_D^{25}$ +128° (*c* 0·4 in $CHCl_3$) (Found: C, 66·2; H, 6·2; N, 20·0; *C*-Me, 3·8. $C_{23}H_{26}N_6O_2$ requires C, 66·1; H, 6·2; N, 20·3; 1*C*-Me, 3·6%), $\lambda_{max.}$ 248 (log ε 4·5) and 286 mμ (log ε 4·5). The residual solution from steam-distillation was neutralised with sodium carbonate and evaporated to dryness. Extraction of the residue with methanol gave a gum which yielded β-glucose penta-acetate, m. p. and mixed m. p. 134—135°.

Tetrahydrodideoxyaucubigenin 2,4-Dinitrophenylhydrazone (XI).—Tetrahydrodideoxyaucubin tetra-acetate, m. p. 91° (50 mg.), was refluxed under nitrogen with N-sodium hydroxide (5 ml.) for 30 min., then tartaric acid (2 g.) in water was added and the mixture steam-distilled. From the distillate 2,4-dinitrophenylhydrazine in dilute hydrochloric acid slowly precipitated 2,4-dinitrophenylhydrazone which was collected after 12 hr. Crystallisation from aqueous ethanol afforded orange needles, m. p. 129—132° (Found: C, 52·9; H, 5·9; N, 16·1. Calc. for $C_{15}H_{20}N_4O_5$: C, 53·4; H, 5·9; N, 16·5%). This is probably the stereoisomer obtained by the Japanese authors [1] who give m. p. 134—136°.

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