668. *The Chemistry of the* Aristolochia *Species. Part IV.* The Structure of Aristolactone.*

By J. W. Steele, J. B. Stenlake, and W. D. Williams.

The sesquiterpenoid lactone, aristolactone, is shown to be $\beta\gamma$-unsaturated. Oxidation by chromic acid to succinic and acetic acid, and dehydrogenation of aristolactone derivatives to vetivazulene and a naphthalenic substance, led to the monocarbocyclic structure (I). This is supported by the properties of methyl oxoaristate and other aristolactone derivatives.

In reporting our further work on aristolactone, we mention first a few miscellaneous observations. The yields of aristolactone from *Aristolochia reticulata* have been improved by chromatographing the petrol-soluble extract which remains after crystallisation of the lactone. The lactone has also been isolated from *A. serpentaria* Linn., but is not found in either *A. longa* Linn. or *A. indica* Linn. The re-arrangement of aristolactone to isoaristolactone described in Part II [1] has been re-examined: heating with a strong sulphonic acid resin in ethanol gave improved yields, and almost quantitative yields were obtained when aristolactone was treated with warm ethanolic sulphuric acid.

In our structural work we found ozonolysis of aristolactone and isoaristolactone to give formaldehyde (as dimedone derivative) in yields of 29% and 26·5%, respectively, based on two vinylidene substituents, compared with 36% for ethyl oxoaristate based on a single vinylidene substituent. The dihydro-lactone obtained from both aristolactone and isoaristolactone failed to yield formaldehyde on ozonolysis, indicating reduction and re-arrangement, respectively, of the vinylidene groups. The dihydro-lactone is now regarded as an isodihydroisoaristolactone, and not dihydroaristolactone as previously designated, since (*a*) its slow hydrolysis by ethanolic potassium hydroxide is characteristic of isoaristolactone and in contrast with that of aristolactone, (*b*) isodihydroisoaristolactone has $[\alpha]_D^{17}$ −77°, which is comparable with that of isoaristolactone ($[\alpha]_D^{17}$ −44°) and in contrast to that of aristolactone ($[\alpha]_D^{14}$ +156·4°), and (*c*) infrared spectra show a trisubstituted double bond (band at 815 cm.$^{-1}$ in carbon disulphide) in both isoaristolactone and isodihydroisoaristolactone, not evident with aristolactone. Hydrogenation of one vinylidene group in isoaristolactone is, therefore, accompanied by re-arrangement of the second vinylidene group to a trisubstituted ethylenic group, and reduction of aristolactone to isodihydroisoaristolactone is to be explained by re-arrangement of both the " lactone double bond " and the remaining vinylidene group. Similar re-arrangements are known,[2] and the steric requirements of such migrations induced by hydrogenation catalysts have been discussed.[3] The present conclusions are also supported by evidence of similar transformations in the reduction of the sesquiterpene reticulene,[4] which occurs with aristolactone in *A. reticulata* and *A. serpenteria*. Reticulene, which possesses two vinylidene substituents,

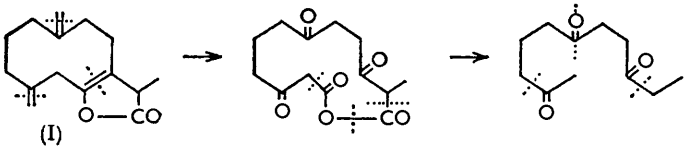
* Part III, *J.*, 1957, 4120.

[1] Stenlake and Williams, *J.*, 1955, 2114.
[2] Irvine, Laurie, McNab, and Spring, *J.*, 1956, 2029; Howe and McQuillin, *J.*, 1956, 2670.
[3] Bream, Eaton, and Henbest, *J.*, 1957, 1974.
[4] Stenlake and Williams, *J. Pharm. Pharmacol.*, 1954, **6**, 1005.

yields dihydroisoreticulene on partial hydrogenation, by reduction of one vinylidene and re-arrangement of the other to a trisubstituted ethylenic group.[5]

Assignment of the lactone double bond as αβ was based on the assumption now disproved (above) that isodihydroisoaristolactone was formed by reduction of the lactone double bond, and not the vinylidene group. Re-examination of the ultraviolet absorption spectrum of aristolactone and isoaristolactone down to 198 mμ (a fused silica prism being used), and other evidence described below establish the lactone ring as βγ-unsaturated. This does not conflict with our interpretation of the alkaline re-arrangement to methyl and ethyl oxoaristate and, further, provides a more satisfactory explanation of the fractional iodine values found for aristolactone [1] (cf. Cavallito and Haskell [6]). It also explains the failure of aristolactone to respond to the Legal test, and its failure to form an ammonia adduct.[1] Hydrogenolysis with the formation of acidic products during hydrogenation of aristolactone in acid solution (hydrogen uptake 4 mols.) also supports the βγ-unsaturated γ-lactone formulation.[7]

The carbon skeleton of aristolactone, for which we now propose structure (I), was suggested by dehydrogenation with palladium–charcoal. Experiments with aristolactone, isohexahydroisoaristolactone,* and other crystalline derivatives failed, but the oily products remaining after the preparation of isohexahydroisoaristolactone, dihydroxyaristolactone, isoaristolactone, and methyl oxoaristate (all from pure crystalline lactone) all gave a violet azulene (in poor yield) and, in one instance, a trace of a naphthalenic derivative. The ultraviolet and visible absorption spectra of the azulene and of its azulenium ion (in 50% sulphuric acid) were virtually identical with the corresponding spectra of vetivazulene (2-isopropyl-4,8-dimethylazulene)[8]. Although formation of vetivazulene from uncharacterised intermediates is inconclusive, the preparation of these intermediates, in every case from either pure crystalline aristolactone or crystalline lactone derivatives, implies an element of skeletal relation between the latter and vetivazulene. Production of vetivazulene and naphthalenic products from monocyclic parent structures does not exclude skeletons of the elemol [9] (III) and possibly also the xanthanin type (IV), though no aromatic dehydrogenation products have, in fact, been obtained from xanthinin.[10] But, considered with the other evidence (below), it suggests that structure (I), based on a cyclodecane skeleton which conforms to the isoprene rule, offers the most satisfactory explanation of the properties of aristolactone.



(I)

In support of structure (I), vigorous oxidation of aristolactone with potassium dichromate and sulphuric acid gave formic acid (trace) and acetic acid (1·6 mol.); the non-volatile residue gave succinic acid, confirmed as its *S*-benzylthiuronium salt, in agreement with the annexed degradation of structure (I), which, despite the presence of only one *C*-methyl group, permits the formation of 2 mol. of acetic acid. Paper chromatography

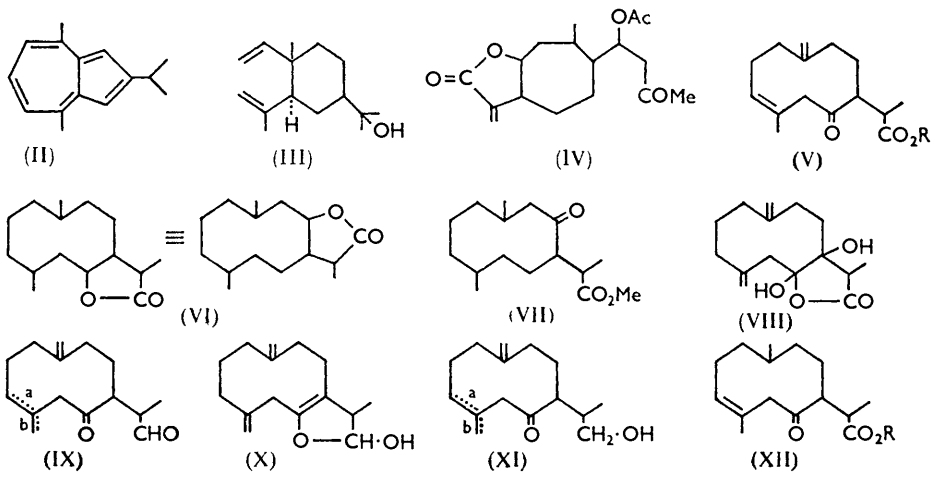
* The change in nomenclature from hexahydroaristolactone to isohexahydroisoaristolactone follows from the fact that it is formed from isodihydroisoaristolactone.

[5] Coutts, Stenlake, and Williams, unpublished work.
[6] Cavallito and Haskell, *J. Amer. Chem. Soc.*, 1946, **68**, 2332.
[7] Jacobs and Scott, *J. Biol. Chem.*, 1930, **87**, 601; 1931, **93**, 139.
[8] Susz, Pfau, and Plattner, *Helv. Chim. Acta*, 1937, **20**, 469; Sörensen and Hougen, *Acta Chem. Scand.*, 1948, **2**, 447; Chopard-dit-Jean and Heilbronner, *Helv. Chim. Acta*, 1952, **35**, 2170.
[9] Sykora, Hérout, and Šorm, *Coll. Czech. Chem. Comm.*, 1955, **20**, 220.
[10] Geissman and Deuel, *Chem. and Ind.*, 1957, 328; *J. Amer. Chem. Soc.*, 1957, **79**, 3778; Geissman, Deuel, Bonde, and Addicott, *ibid.*, 1954, **76**, 685; Dolejs, Hérout, and Šorm, *Coll. Czech. Chem. Comm.*, 1958, **23**, 504.

of the mother-liquors in a phenol–formic acid system gave well-defined spots of succinic acid, and showed the complete absence of glutaric and higher dibasic acids, as expected.

Evidence for three double bonds in aristolactone (I) rests on reduction of the lactone and of methyl oxoaristate (V; R = Me) to isohexahydroisoaristolactone (VI) and the oily methyl tetrahydro-oxoaristate (VII) respectively, and on the determination of bromine numbers.[1] Re-determination of bromine numbers for aristolactone, its derivatives, and a series of control compounds (Table 2, p. 3299) shows that both reaction time and solvent are critical. Unhindered double bonds react quantitatively in carbon tetrachloride within two minutes (compounds 1 and 2) but, under the same conditions, hindered double bonds give fractional values (compound 5), whilst cyclopropane rings undergo negligible reaction (compounds 6 and 7). Results for 6,7-dihydroxyaristolactone (VIII) (compound 10) and iso-oxoaristaldehyde (IX) (compound 11), which lack the hindered lactone double bond of aristolactone, indicate two double bonds. Aristolactone (I) reacts with 2·6 mol. of perbenzoic acid in 48 hr., in conformity with the presence of three double bonds.

The lactone double bond was confirmed as $\beta\gamma$ by reduction of aristolactone (I) with lithium aluminium hydride, which gave two products depending upon the conditions. At 0° a crystalline product was obtained, which is probably the γ-keto-aldehyde (IX), $[\alpha]_D$ +82°, formed by re-arrangement of the intermediate hemiacetal [11] (X). Compound (IX) gave a positive Schiff's reaction, reduced ammoniacal silver nitrate (aldehyde), and showed end-absorption in the ultraviolet region (ε 3234 at 210 mμ) with a secondary band of absorption and low-intensity maximum at 284 mμ (ε 58; aldehyde and ketone). The infrared spectrum (potassium bromide disc) showed bands confirming the vinylidene absorption (904 and 1645 cm.$^{-1}$) and a single broad band in the carbonyl stretching region with a peak at 1752 cm.$^{-1}$ (1746 cm.$^{-1}$ in chloroform). The latter band, unlike the carbonyl bands of methyl oxoaristate, is not resolved, and, moreover, is not typical of the proposed oxocyclodecan-aldehyde structure. The reason for this anomaly is not clear and further investigations are in prospect. More vigorous reduction with lithium aluminium hydride gave the 1,4-ketol, oxoaristoöl (XI), which showed end-absorption in the ultraviolet spectrum [ε 3860 at 210 mμ (vinylidene), and a shoulder at 280 mμ (ketone)].



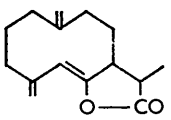
On the basis of structure (I), methyl and ethyl oxoaristate, the products of re-arrangement with cold methanolic or ethanolic alkali,[1] can be assigned structures (V; R = Me and Et, respectively). Ozonolysis of ethyl oxoaristate gave formaldehyde, confirming the presence of a vinylidene group, whilst a trisubstituted double bond is indicated by an infrared band at 815 cm.$^{-1}$ not present in aristolactone. Careful hydrogenation of methyl

[11] Arth, *J. Amer. Chem. Soc.*, 1953, **75**, 2413; Dietrich, Lederer, and Mercier, *Annalen*, 1957, **603**, 8.

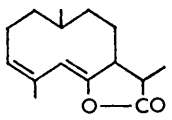
oxoaristate at a platinum catalyst gave, after the uptake of 1 mol., a new crystalline γ-keto-ester, methyl dihydro-oxoaristate (XII; R = Me), in which the vinylidene group had been reduced, as shown by the absence of formaldehyde after ozonolysis.

Reduction of the vinylidene group of methyl oxoaristate is accompanied by a marked fall in absorption intensity at 209 mμ (Δε 4040). Comparable Δε values for the reduction of ethyl oxoaristate (V; R = Et) to ethyl dihydro-oxoaristate (XII; R = Et), and of isoaristolactone (XIII) to isodihydroisoaristolactone (XIV) are 3430 and 3400 respectively. The last two reductions both concern vinylidene groups, since neither product yields formaldehyde on ozonolysis. This unexpectedly large vinylidene contribution to the end-absorption invalidates the previous assumption [1] of a tetrasubstituted double bond in methyl oxoaristate. The presence of a trisubstituted double bond in methyl dihydro-oxoaristate has now been demonstrated by (*a*) a yellow colour with tetranitromethane, (*b*) catalytic reduction to methyl tetrahydro-oxoaristate (VII) (hydrogen uptake 1 mol.), (*c*) end-absorption in the ultraviolet (ε 2300 at 209 mμ), (*d*) an infrared band at 815 cm.$^{-1}$ (potassium bromide disc), and (*e*) ozonolysis to a product having a positive nitroprusside reaction for methyl ketone (not given by methyl dihydro-oxoaristate), which, however, was not the expected diketonic acid, $C_{16}H_{26}O_6$, but instead a weakly acidic substance, $C_{16}H_{24}O_5$, formed apparently by loss of the elements of water during distillation. This product, which was not characterised, showed pH- and concentration-dependent absorption in the ultraviolet region (Table 1, p. 3297). Its formation, however, without loss of carbon establishes the ozonised double bond as part of a cyclic system.

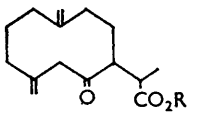
It might be expected, by analogy with the reduction of isoaristolactone (XIII) to isodihydroisoaristolactone (XIV), that the trisubstituted double bond in methyl dihydro-oxoaristate (XII; R = Me) also arises from a methyl oxoaristate of structure (XV) by re-arrangement at the reduction stage. Both methyl and ethyl oxoaristate, however, show an infrared band at 815 cm.$^{-1}$ (in carbon disulphide), in support of the alternative structure (V). Moreover, the divinylidene structure (XV) is excluded further by a comparison of molecular models. These reveal the improbability of a ten-membered ring with three exocyclic multiple bonds, for which the only conformations are those with an unduly high proportion of unfavourable methylene conformations. In contrast, the virtually



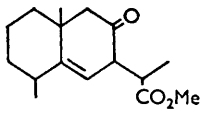
(XIII)



(XIV)



(XV)



(XVI)

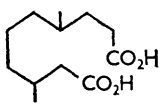
strainless conformation(s) of structure (V) with one endocyclic and two exocyclic multiple bonds would provide the necessary driving force for the further re-arrangement which is now implied in the formation of the oxoaristates (V) from aristolactone (I). Similar considerations apply also to oxoaristaldehyde (VI), iso-oxoaristaldehyde (IX; see below), and aristoöl (XI), and suggest that the structures (IXa) and (XIa) are preferable to the alternatives (b) although no direct evidence is available.

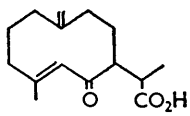
The carbonyl groups in both methyl and ethyl oxoaristate (V; R = Me and Et) showed the typical non-reactivity expected of cyclodecanones,[12] failing to form an oxime, semicarbazone, or 2,4-dinitrophenylhydrazone. Methyl dihydro- (XII; R = Me) and tetrahydro-oxoaristate (VII) also failed to react with carbonyl reagents. Attempts to reduce the carbonyl group of methyl tetrahydro-oxoaristate with aluminium isopropoxide were also unsuccessful, and consistently the carbonyl group in oxoaristaldehyde (IX) resisted reduction with lithium aluminium hydride under normal conditions. That the non-reactivity of these compounds is due to an " O-inside " conformation of the cyclodecane carbonyl group rather than hindrance of the adjacent methyl and methoxycarbonyl

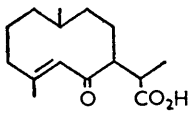
[12] Prelog, *J.*, 1950, 420.

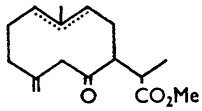
substituents is established by comparison with the analogous methyl 8-oxosanta-5-enoate [13,14] (XVI) which readily forms a crystalline 2,4-dinitrophenylhydrazone.[13] Further, methyl dihydro-oxoaristate (XII; R = Me) shows a carbonyl band in the infrared spectrum at 1693 (in potassium bromide) and 1698 cm.$^{-1}$ (in chloroform) typical of large-ring ketones.[15] Methyl oxoaristate similarly shows carbonyl absorption at 1693 cm.$^{-1}$ (in potassium bromide).

Ring cleavage of methyl tetrahydro-oxoaristate (VII) by nitric acid gave a saturated (and therefore acyclic) dibasic acid $C_{12}H_{22}O_4$, isolated as the silver salt. Although not characterised as the expected 4,8-dimethylsebacic acid (XVII), this product is consistent with the large-ring ketone structure. Moreover, paper chromatography of the oily oxidation product showed no evidence of small fragments, such as α-methyladipic acid, which might be expected to arise from a cyclohexane derivative.


(XVII)


(XVIII)


(XIX)


(XX)

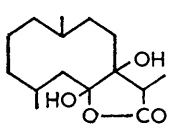
The two non-lactonic double bonds in aristolactone (I) were located by the action of alkali on methyl oxoaristate (V; R = Me) and methyl dihydro-oxoaristate (XII; R = Me). The former, when refluxed with methanolic potassium hydroxide, gave a crystalline iso-oxoaristic acid (XVIII), $[\alpha]_D$ −3·45°, which exhibited an ultraviolet absorption maximum at 243 mμ (ε 6780), in agreement with the calculated wavelength (239 mμ) for an αβ-unsaturated ketone with two β-substituents.[16] Methyl dihydro-oxoaristate (XII; R = Me), treated similarly with alkali, gave an oily product (XIX), which also showed an ultraviolet absorption maximum at 245 mμ (ε 6300). This confirms that it is the trisubstituted double bond and not the vinylidene group of methyl oxoaristate that is βγ to the carbonyl group, and thereby excludes the alternative structures (XX) for methyl oxoaristate.

Aristolactone (I) with potassium permanganate in acetone gave 6,7-dihydroxyaristolactone (VIII). The latter is doubly unsaturated (bromine number 1·90), is readily hydrogenated to tetrahydrodihydroxyaristolactone (XXI), and exhibits end-absorption in the ultraviolet region (ε 3200 at 210 mμ) consistent with retention of the vinylidene double bonds. Both 6,7-dihydroxyaristolactone and its tetrahydro-derivative show an infrared band at 1767 cm.$^{-1}$ (in chloroform) typical of a saturated γ-lactone. The 1,2-glycol link was demontrated in tetrahydro-6,7-dihydroxyaristolactone (XXI) by oxidation with sodium bismuthate,[17] although the oily product gave only an amorphous 2,4-dinitrophenylhydrazone which was unstable and resisted characterisation. 6,7-Dihydroxyaristolactone (VIII) showed an infrared band at 906 cm.$^{-1}$ (in potassium bromide; vinylidene), failed to yield formaldehyde with sodium metaperiodate,[18] and did not re-arrange in acid solution (see below). A second product of the permanganate oxidation of aristolactone, an acidic oil, gave succinic acid as the only characterisable product when the oxidation was continued.

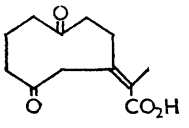
Acid-catalysed re-arrangement of aristolactone (I) to isoaristolactone (XIII), which results from migration of the lactone double bond,[1] is seen as a shift from a less stable βγ-endocyclic position to the more stable γδ-exocyclic position.[19] Conversion of iso-aristolactone by lithium aluminium hydride at 0° into iso-oxoaristaldehyde, $[\alpha]_D$ −50·1°,

[13] Ukita and Nakazawa, *Pharm. Bull.* (*Japan*), 1954, **2**, 299.
[14] Asselineau and Bory, *Compt. rend.*, 1958, **246**, 1874.
[15] Šorm, Dolejs, and Pliva, *Coll. Czech. Chem. Comm.*, 1950, **15**, 186.
[16] Woodward, *J. Amer. Chem. Soc.*, 1941, **63**, 1123; 1942, **64**, 76.
[17] Rigby, *J.*, 1950, 1907.
[18] Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476.
[19] Brown, Brewster, and Shechter, *ibid.*, 1954, **76**, 467.

stereoisomeric with oxoaristaldehyde (IX), establishes the direction of double-bond shift to be as indicated. The stability of 6,7-dihydroxyaristolactone to acid under similar conditions (above) not only identifies the locus of re-arrangement, but also establishes that this is confined to a shift of the lactone double bond. Oxidation of isoaristolactone with chromic acid yields succinic acid as the largest identifiable fragment in accordance



(XXI)



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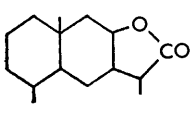

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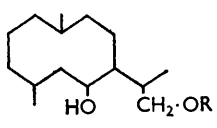

(XXIV)

with structure (XIII), but the ultraviolet absorption spectrum does not show the expected intense maximum for the postulated diene system.[20] Molecular models, however, reveal that the two double bonds are non-coplanar in the only strainless conformations for the 1,3-diene system of isoaristolactone and for the cyclodeca-1,3-diene of isodihydroisoaristolactone, and depression of absorption intensities to the small maxima observed [1] at 272 mμ in isoaristolactone (ε 640) and isodihydroisoaristolactone (ε 606) accords with this lack of coplanarity in the diene system.[21] The diene structure is supported by the significantly greater end-absorption of isoaristolactone (ε 16,280 at 204 mμ) compared with that of aristolactone (ε 12,000 at 206 mμ), when examined with fused silica optics.

Ozonolysis of aristolactone gave formaldehyde as the sole volatile product. Decomposition of the ozonide with water or zinc and glacial acetic acid, or by catalytic reduction, gave acidic oils which were rapidly discolored by alkali, even at room temperature. Paper chromatography of the acidic product with (*a*) butan-1-ol–pyridine–water–ethanol and (*b*) pyridine–water–ammonia showed the presence of two keto-acids A [R_F 0·62 in (*a*) and 0·80 in (*b*)] and B [R_F 0·31 in (*a*) and 0·45 in (*b*)]. Oxidative hydrolysis of the ozonide gave the oily unsaturated ketonic acid, B, which showed end-absorption ($E^{1\%}_{1\,cm.}$ 250 at 210 mμ) and saturated ketone absorption at 275 mμ ($E^{1\%}_{1\,cm.}$ 5·6). The product gave no ferric chloride reaction, was readily decarboxylated on distillation to an unsaturated neutral oil (which showed end-absorption in the ultraviolet spectrum and gave analyses approximating to $C_{11}H_{16}O_2$), and underwent rapid ozonolysis to acetaldehyde (identified as its 2,4-dinitrophenylhydrazone and dimedone derivatives), in accord with the tentative structure (XXII).

The close structural relation between isohexahydroisoaristolactone (VI) and tetrahydroalantolactone [22] (XXIII) is borne out by a remarkable similarity in the infrared spectrum of the two compounds. We have, however, been unable to establish a link with the fully saturated lactone also of structure (VI) obtained by Suchy, Horak, Hérout, and Šorm,[23] by degradation of arctiopicrin. This lactone, derived from a crystalline keto-lactonic precursor, was liquid, and no optical rotation was reported, so that comparison with the presumed stereoisomeric isohexahydroisoaristolactone was not possible. Reduction of the liquid lactone with lithium aluminium hydride was reported to give a crystalline diol, m. p. 117° (XXIV; R = H), the monobenzoate (m. p. 89°) of which yielded, on oxidation, the corresponding 6-keto-12-benzoate for which no characteristics other than the infrared carbonyl frequency were given.[23] Similar reduction of isohexahydroisoaristolactone, however, afforded an almost quantitative yield of an isomeric crystalline diol, tetrahydroisoaristo-6,12-diol, m. p. 106—107°, $[\alpha]_D$ +18·7°, the 12-monobenzoate (XXIV; R = Bz) of which was obtained only as an oil, $[\alpha]_D$ +1·9°. No definite conclusion can

[20] Fieser and Fieser, " Natural Products Related to Phenanthrene," 3rd edn., Reinhold Publ. Corp., 1949, p. 185.
[21] Braude and Nachod, " Determination of Organic Structures by Physical Methods," Academic Press Inc., 1955, p. 169; Braude and Gofton, *J.*, 1957, 4270.
[22] Tsuda, Tanabe, Iwai, and Funakoshi, *J. Amer. Chem. Soc.*, 1957, **79**, 1009.
[23] Suchy, Horak, Hérout, and Šorm. *Chem. and Ind.*, 1957, 894.

therefore be reached concerning the identity or non-identity of the two lactones and their derivatives.

Stereochemical relations of aristolactone derivatives will be discussed in a later paper.

Experimental

Unless otherwise stated, rotations refer to solutions in absolute ethanol in a 1 dcm. tube, and ultraviolet absorption spectra to solutions in absolute ethanol, determined on a Hilger Uvispek spectrophotometer. We are indebted to Mr. W. McCorkindale and Dr. A. C. Syme for the microanalyses.

Aristolactone.—(*a*) This was isolated from the light petroleum-soluble extract of *Aristolochia reticulata* by seeding and crystallisation as described in Part I.[4] Chromatography of the residual oil in light petroleum (b. p. 40—60°) on a column of mixed activated charcoal (1 part) and Whatman standard grade cellulose powder (3 parts), and elution with the same solvent, gave a small lævorotatory fraction, followed by a strongly dextrorotatory fraction, from which a further yield of aristolactone, m. p. 110—111°, crystallised (total yield, 15 g. from 9·5 kg.).

(*b*) Extraction of *Aristolochia serpentaria* Linn. (4·5 kg.) as described above yielded 7·9 g. of aristolactone.

(*c*) Perbenzoic acid titration, showed double-bond equivalents of 2·28 (after 6 hr.), 2·56 (after 24 hr.), and 2·63 (after 48 hr.).

Ozonolysis of Aristolactone.—(*a*) Aristolactone (1·64 g.) was ozonised in dry chloroform (30 ml.) at 0° for 3 hr. The solvent was evaporated at room temperature, and the glassy ozonide refluxed for 3 hr. with 0·1N-hydrochloric acid (10 ml.), 30% hydrogen peroxide (10 ml.), and water (30 ml.). The oily suspension was extracted with chloroform, and the latter dried (Na_2SO_4) and evaporated to give a pale yellow acidic oil (0·83 g.) (Found: equiv., 224), $\lambda_{max.}$ 275 mμ ($E^{1\%}_{1\,cm.}$ 5·6), end absorption at 210 mμ ($E^{1\%}_{1\,cm.}$ 250).

The aqueous liquor, which gave a strongly positive sodium nitroprusside reaction for methyl ketone, was concentrated by freeze-drying, neutralised with sodium hydroxide, and distilled. The distillate gave an orange 2,4-dinitrophenylhydrazone, which when chromatographed on alumina gave two small unidentified fractions, m. p. 162—164° and 152—154° severally.

Ozonolysis of the chloroform-soluble oil from the above ozonolysis for a further 1½ hr., with decomposition and extraction as described, gave a viscous oil (0·25 g.). Distillation of the remaining aqueous liquors into an acid 50% ethanolic solution of 2,4-dinitrophenylhydrazine yielded acetaldehyde 2,4-dinitrophenylhydrazone, m. p. 147°, undepressed on admixture with authentic material (m. p. 149°) (Found: C, 42·7; H, 3·2; N, 24·9; O, 29·1. Calc. for $C_8H_8O_4N_4$: C, 42·9; H, 3·6; N, 25·0; O, 28·5%). Distillation into saturated aqueous dimedone gave acetaldehyde dimedone derivative, m. p. 131—134°, mixed m. p. with authentic material 132—135°.

(*b*) Aristolactone was ozonised in chloroform, the ozonide decomposed with hydrochloric acid and hydrogen peroxide as described above, and the oily fraction distilled to yield a colourless neutral oil (Found: C, 73·8; H, 10·1. $C_{11}H_{16}O_2$ requires C, 73·3; H, 9·0%). This was presumed to be 7-ethylidenecyclononane-1,5-dione.

(*c*) Aristolactone (415 mg.) was ozonised in chloroform, and the ozonide solution decomposed with zinc powder (0·4 g.) and glacial acetic acid (2 ml.). The aqueous extract was neutralised and finally made faintly acid with acetic acid. Addition of dimedone (0·5 g.) in 50% ethanol (20 ml.) gave formaldehyde–dimedone derivative (308 mg.; 29% calc. for two vinylidene groups), m. p. and mixed m. p. 190—191°.

Isoaristolactone.—(*a*) Aristolactone (0·1 g.) in 95% ethanol (10 ml.) was refluxed with activated Zeo-Karb-225 (1 g.) until the optical rotation had reached a constant negative value (*ca.* 9 hr.). Evaporation of the solvent gave isoaristolactone, m. p. 90—91° (35 mg.; from light petroleum).

(*b*) Aristolactone (50 mg.) in 99% ethanol (3 ml.) was mixed with a 10% solution (1 ml.) of sulphuric acid in 50% ethanol, warmed to 40° for 2 min., and allowed to cool. The crystalline product, obtained by adding water dropwise, gave isoaristolactone (40 mg.; from aqueous ethanol), m. p. 89·5—90° (block), $[\alpha]_D^{20}$ −42°.

(*c*) Aristolactone (490 mg.) in 99% ethanol (10 ml.) was mixed with a 10% solution (40 ml.) of sulphuric acid in 50% ethanol at room temperature and the reaction followed polarimetrically. When the optical rotation reached a constant negative value (5 hr.), dilution with water gave

colourless needles of isoaristolactone (430 mg.; 86% from aqueous ethanol), m. p. 90—91° (block), $\nu_{max.}$ (in potassium bromide disc) at 1742 (unsaturated γ-lactone), 902 and 1641 (vinylidene), and 824 cm.$^{-1}$.

Ozonolysis of Isoaristolactone.—Isoaristolactone (153 mg.) was ozonised in chloroform (15 ml.) at −7°, and the solution treated with glacial acetic acid (2 ml.) and zinc powder (0·4 g.), added gradually (10 min.). The solution was filtered, extracted with water (4 × 20 ml.), neutralised, and treated with dimedone (350 mg.) in ethanol (20 ml.), to give formaldehyde–dimedone derivative (103 mg., 26·5% calc. yield for two vinylidene groups), m. p. and mixed m. p. 183—187°.

Ozonolysis of Isodihydroisoaristolactone.—Isodihydroisoaristolactone (176 mg.) was ozonised in chloroform (5 ml.), and the ozonide decomposed as described for isoaristolactone. The aqueous extract gave no precipitate when treated with dimedone or when distilled into an acid solution of 2,4-dinitrophenylhydrazine.

Chromic Acid Oxidation of Aristolactone.—Aristolactone (476 mg.) was warmed cautiously with a cooled mixture of potassium dichromate (4·5 g.), sulphuric acid (4 ml.) and water (10 ml.). After reaction had subsided, the mixture was refluxed gently for 2 hr., then steam-distilled. The distillate required 5·8 ml. of 0·5N-sodium hydroxide for neutralisation (equiv. to 1·6 mol.) and gave crystalline sodium acetate on evaporation, identified by ferric chloride reaction (red) and by conversion into *S*-benzylthiuronium acetate, m. p. 141—142° (from aqueous ethanol) undepressed on admixture with authentic material, m. p. 143—144°.

Non-volatile products were extracted with ether (6 × 50 ml.), and the extracts were dried (Na_2SO_4) and evaporated to yield a semicrystalline residue. Recrystallisation from light petroleum–ethanol and then ether gave succinic acid, m. p. and mixed m. p. 183—185°. The acid gave *S*-benzylthiuronium succinate, m. p. and mixed m. p. 153—154°.

The residue from the mother-liquors was chromatographed in No. 1 Whatman filter paper by the ascending technique, with phenol–formic acid–water (80 : 1 : 19), and the paper was sprayed with Bromocresol Green. Drying showed two yellow (acidic) spots, of R_F 0·71 (succinic acid, as shown by comparison with an authentic sample) and R_F 0·99. The latter acid was not identified, but was shown to possess a carbonyl group by spraying with an acid solution of 2,4-dinitrophenylhydrazine.

Chromic Acid Oxidation of Isoaristolactone.—Isoaristolactone (500 mg.) was gently refluxed for 2 hr. with potassium dichromate (4·5 g.), water (10 ml.) and sulphuric acid (4 ml.). Steam-distillation gave acetic acid. Non-volatile products were extracted with ether, and the ethereal solution was evaporated. The residue, washed with chloroform, yielded succinic acid, m. p. 183—185°, R_F 0·75 (phenol–formic acid–water, 80 : 1 : 19). The mother-liquors were partitioned in chloroform on a buffered (pH 7·4) silica gel column to yield an acidic oil, R_F 0·97 (phenol–formic acid–water, 80 : 1 : 19), R_F 0·90 (butan-1-ol–formic acid–water, 10 : 3 : 10); distillation gave a semicrystalline product which could not be characterised.

Oxoaristaldehyde (IX).—Aristolactone (520 mg.) was added portionwise to lithium aluminium hydride (125 mg.) in sodium-dried ether (15 ml.) at 0° during 10 min. After a further 5 min. the solution was allowed to warm to room temperature. Excess of reagent was destroyed by water (1 drop), and the solution treated with dilute hydrochloric acid and extracted with ether. The ethereal solution, when dried (Na_2SO_4) and evaporated, gave an oil (500 mg.) which gave colourless needles of *oxoaristaldehyde*, m. p. 197—198° (block) (from acetone), $[\alpha]_D^{16\cdot5}$ +82° (*c* 0·48), $\lambda_{max.}$ 284 (ε 58), end-absorption at 210 mμ (ε 3234) (Found: C, 76·9; H, 9·25. $C_{15}H_{22}O_2$ requires C, 76·9; H, 9·5%). This reduced ammoniacal silver nitrate and restored the colour to Schiff's reagent.

Attempted Isomerisation of Oxoaristaldehyde.—Oxoaristaldehyde (90 mg.) was dissolved in a 10% solution (8 ml.) of sulphuric acid in 50% ethanol, and the optical rotation observed during 6 hr. No change was observed, and oxoaristaldehyde, m. p. 197—198°, was recovered.

Oxoaristoöl.—Aristolactone (120 mg.) was added portionwise during 10 min. to lithium aluminium hydride (39 mg.) in dry ether (5 ml.) at room temperature, and the mixture refluxed gently for 5 min. Excess of reagent was destroyed by water, and the solution treated with dilute hydrochloric acid, and extracted with ether. The ethereal solution was dried (Na_2SO_4) and evaporated, yielding a colourless oil, which crystallised from acetone to give colourless needles of *oxoaristoöl* (10 mg.), m. p. 245—246°, $\lambda_{max.}$ 270—275 mμ (ε about 184), end-absorption at 210 mμ (ε 3860) (Found: C, 76·5; H, 10·0. $C_{15}H_{24}O_2$ requires C, 76·3; H, 10·2%).

Iso-oxoaristaldehyde (IX).—Isoaristolactone (400 mg.) in sodium-dried ether (25 ml.) was

reduced with lithium aluminium hydride (60 mg.) as described for aristolactone; it yielded *iso-oxoaristaldehyde* (40 mg.; from methanol), m. p. 211—213° (block), $[\alpha]_D^{20}$ −50·1° (*c* 0·40 in chloroform), $\lambda_{max.}$ 290 mμ (ε 30), end-absorption at 209 mμ (ε 4960) (Found: C, 77·4; H, 9·3. $C_{15}H_{22}O_2$ requires C, 76·9; H, 9·5%), $\nu_{max.}$ 1753 cm.$^{-1}$ (C=O) in chloroform. This aldehyde reduced warm ammoniacal silver nitrate and restored the colour to Schiff's reagent.

Methyl Dihydro-oxoaristate.—Methyl oxoaristate (1 g.) in ethanol (25 ml.) was shaken with platinum oxide (0·1 g.) in presence of hydrogen, until 1 mol. was absorbed. Filtration and evaporation gave colourless *methyl dihydro-oxoaristate* (0·9 g.), m. p. 68—68·5° (block) (from 60% ethanol), $[\alpha]_D^{15}$ +152° (*c* 0·788), $\lambda_{max.}$ 290 mμ (ε 160), end-absorption at 210 mμ (ε 3600), $\nu_{max.}$ (in KBr disc) 815 (trisubstituted double bond), 1732 (ester C=O) and 1693 cm.$^{-1}$ (ketone), (in chloroform) 1728 (ester C=O) and 1698 cm.$^{-1}$ (ketone) (Found: C, 71·8; H, 9·8. $C_{16}H_{26}O_3$ requires C, 72·1; H, 9·8%). The product gave a yellow colour with tetranitromethane.

Ethyl Dihydro-oxoaristate.—Ethyl oxoaristate (281 mg.), hydrogenated as above, gave colourless needles of *ethyl dihydro-oxoaristate*, m. p. 65—66° (from ethanol, after sublimation), $[\alpha]_D^{17}$ +131° (*c* 1·21), $\lambda_{max.}$ 287 mμ (ε 52), end-absorption at 208 mμ (ε 3570) (Found: C, 72·8; H, 10·1. $C_{17}H_{28}O_3$ requires C, 73·2; H, 10·2%), giving a yellow colour with tetranitromethane.

Ozonolysis of Ethyl Oxoaristate.—Ethyl oxoaristate (290 mg.) in chloroform (10 ml.) was treated with ozonised oxygen at 0°. The ozonide obtained on evaporation of the solvent was decomposed by the addition of water. The solution was kept overnight, then distilled, and the distillate was treated with dimedone (500 mg.) in 30% ethanol (20 ml.) to give formaldehyde–dimedone derivative (112 mg., 36% calc. on one vinylidene group), m. p. and mixed m. p. 188—190°. Distillation of the aqueous filtrate gave a small amount of a 2,4-dinitrophenylhydrazone, m. p. 130—134° (from ethanol), insufficient for characterisation. The residual non-volatile oil (260 mg.) had equiv. 228 and $\lambda_{max.}$ 280 mμ ($E_{1\,cm.}^{1\%}$ 14·2), and gave positive reactions with alkaline sodium nitroprusside (methyl ketone) and ammoniacal silver nitrate, but was not characterised.

Ozonolysis of Ethyl Dihydro-oxoaristate.—Ethyl dihydro-oxoaristate (100 mg.) was ozonised as described for isoaristolactone. The resulting aqueous solution gave no colour with Schiff's reagent and no precipitate with dimedone. The oily residue from the chloroform solution gave a positive reaction with alkaline sodium nitroprusside for methyl ketone.

Ozonolysis of Methyl Dihydro-oxoaristate.—Methyl dihydro-oxoaristate (1·37 g.) was ozonised as described for methyl oxoaristate. The aqueous distillate failed to react with 2,4-dinitrophenylhydrazine. The non-volatile residue extracted with chloroform and distilled gave a pale yellow oil (0·58 g.), b. p. 220—240°/0·4 mm. (Found: C, 65·3; H, 8·6. $C_{16}H_{24}O_5$ requires C, 64·8; H, 8·2%. This product, gave a yellow colour with tetranitromethane (unsaturation), a red colour with alkaline sodium nitroprusside (methyl ketone) and showed the ultraviolet absorptions recorded in Table 1.

The oil (0·27 g.), when treated in 2N-sodium carbonate (20 ml.) at 50° with 3% potassium permanganate solution during 2 hr., gave, after filtration, acidification, and ether-extraction,

TABLE 1.

Concn. (%)	pH	$\lambda_{max.}$	$E_{1\,cm.}^{1\%}$	$\lambda_{max.}$	$E_{1\,cm.}^{1\%}$	Concn. (%)	pH	$\lambda_{max.}$	$E_{1\,cm.}^{1\%}$	$\lambda_{max.}$	$E_{1\,cm.}^{1\%}$
0·0038	1·43	238	250	289	212	0·0038	11·58	249	184	—	—
0·0038	5·80	240	200	289	184	0·00152	5·80	240	164	292	167
0·0038	10·20	249	187	290	167						

a pale yellow oil (0·18 g.). Treatment of the latter with diazomethane yielded a mobile liquid (76 mg.) (Found: C, 67·2; H, 9·4. $C_{15}H_{24}O_4$ requires C, 67·10; H, 9·0%).

Attempted Reduction of Methyl Tetrahydro-oxoaristate.—Methyl tetrahydro-oxoaristate (1·5 g.) in dry propan-2-ol (25 ml.) was refluxed for 6 hr. with aluminium isopropoxide (1 g.). No acetone was produced and methyl tetrahydro-oxoaristate (1·4 g.) was recovered.

Oxidation of Methyl Tetrahydro-oxoaristate.—Methyl tetrahydro-oxoaristate (0·7 g.) in ether was treated with concentrated nitric acid (5 ml.), the mixture being kept at 60° until evolution of nitrous fumes had ceased. Extraction with ether, and treatment of the solution with ammonia, gave an aqueous solution of a dibasic acid, which when chromatographed on Whatman No. 1 paper, with butan-1-ol–formic acid–water (10 : 3 : 10), ran ahead of pimelic acid almost on the solvent front (R_F 0·97). Treatment of the solution with silver nitrate gave colourless *silver 3,7-dimethyldecanedioate* (cf. XVII) (Found: C, 32·6; H, 4·7; Ag, 44·6. $C_{12}H_{20}O_4Ag_2$ requires C, 32·5; H, 4·5; Ag, 48·6%).

Iso-oxoaristic Acid (XVIII).—Aristolactone (460 mg.) in methanol (20 ml.) was treated with cold potassium hydroxide solution (0·5N; 5 ml.) in methanol (90%), the rapidly increasing rotation being observed. On complete conversion into methyl oxoaristate (maximum optical rotation [1]), a further 5 ml. of methanolic potassium hydroxide was added and the solution refluxed for 4 hr. Concentration, acidification, and extraction with ether gave *iso-oxoaristic acid*, as colourless plates (44 mg. from light petroleum), m. p. 143—144° (block), $[\alpha]_D^{20}$ −3·45° (*c* 0·87), $\lambda_{max.}$ 243 mμ (ε 6780) (Found: C, 72·0; H, 8·9. $C_{15}H_{22}O_3$ requires C, 71·8; H, 9·2%).

Dihydroiso-oxoaristic Acid (XIX).—Methyl dihydro-oxoaristate (220 mg.) was refluxed in 0·1N-methanolic potassium hydroxide (25 ml.) for 4 hr. Concentration of the solution, acidification, and extraction with ether gave an oily acidic product (160 mg.), presumed to be dihydroiso-oxoaristic acid, $\lambda_{max.}$ 245 mμ (ε 6300), $[\alpha]_D^{20}$ 0·00°, n_D^{23} 1·5025.

6,7-*Dihydroxyaristolactone*.—Aristolactone (540 mg.) in ice-cold acetone (50 ml.) was treated with potassium permanganate (0·9 g.) in portions during 75 min. The brown precipitate was removed, and the filtrate decolorised with a trace of aristolactone and evaporated to yield a semicrystalline residue (48 mg.). Extraction of the precipitate with hot water, and extraction of the cooled solution with ether, gave further crude material (167 mg.). The product, recrystallised from aqueous ethanol, gave colourless needles of 6,7-*dihydroxyaristolactone*, m. p. 158·5—160°, $[\alpha]_D^{17}$ +128° (*c* 0·39), end-absorption ε 3200 at 210 mμ (Found: C, 68·0; H, 8·55%; Equiv., 272. $C_{15}H_{22}O_4$ requires C, 67·7; H, 8·3%; Equiv., 266).

The aqueous residue remaining after the above extraction with ether was acidified, and the liberated acids were extracted with ether to yield a viscous yellow oil (321 mg.), which gave a red colour with sodium nitroprusside (methyl ketone) but only traces of a flocculent precipitate with 2,4-dinitrophenylhydrazine. The sodium salt of the acid failed to yield a crystalline *S*-benzylthiuronium salt. Oxidation with alkaline potassium permanganate solution, and extraction of the acidic product with ether, gave a semi-crystalline oil, containing succinic acid, identified by partition chromatography on paper with formic acid–phenol–water (1 : 80 : 20) (R_F 0·71).

Periodate Oxidation of 6,7-*Dihydroxyaristolactone*.—Dihydroxyaristolactone (31·3 mg.) was suspended in water (2 ml.), and treated with aqueous N-sodium hydrogen carbonate (1·5 ml.) and 7% aqueous sodium metaperiodate (2 ml.). Dissolution of the lactone was complete in 10 min., but reaction was allowed to proceed for 1½ hr., during which a white precipitate (inorganic) appeared. The solution was filtered and treated with N-hydrochloric acid (3 ml.), 20% aqueous sodium arsenite (2 ml.), aqueous M-sodium acetate (2 ml.), and saturated aqueous dimedone (20 ml.). No precipitation of formaldehyde–dimedone derivative occurred within 2 hr. Similar treatment of milligram quantities of glucose gave quantitative yields of formaldehyde–dimedone.

Attempted Saponification of 6,7-*Dihydroxyaristolactone*.—Dihydroxyaristolactone (200 mg.) in methanol (5 ml.) was refluxed with 0·5N-methanolic potassium hydroxide (3 ml.) for 3½ hr. Neutralisation and extraction with ether yielded unchanged dihydroxyaristolactone (130 mg.). Acidification of the aqueous liquor, extraction with ether, and chromatography from ether on charcoal (5%)–cellulose gave a further 70 mg. of starting material, m. p. 159°. More prolonged treatment with alkali caused some decomposition.

Attempted Isomerisation of 6,7-*Dihydroxyaristolactone*.—Dihydroxyaristolactone (55 mg.) in ethanol (2 ml.) was warmed with a 10% solution of sulphuric acid in 50% ethanol for 2 min. The solution was cooled, diluted with water (10 ml.), and extracted with ether, giving dihydroxyaristolactone (45 mg., from light petroleum–ether), m. p. 159—160° (block), $[\alpha]_D^{18}$ +127°, ε 3300 at 210 mμ.

Tetrahydro-6,7-*dihydroxyaristolactone*.—Dihydroxyaristolactone (90 mg.) in ethanol (10 ml.) was hydrogenated in presence of platinum oxide (12 mg.). Filtration and concentration of the solution gave colourless needles of *tetrahydro*-6,7-*dihydroxyaristolactone* (60 mg. from light petroleum–ether), m. p. 123—124° (block), $[\alpha]_D^{20}$ +32·2° (*c* 0·36 in chloroform) (Found: C, 66·6; H, 9·5. $C_{15}H_{26}O_4$ requires C, 66·7; H, 9·7%).

Sodium Bismuthate Oxidation of Tetrahydro-6,7-*dihydroxyaristolactone*.—Tetrahydro-6,7-dihydroxyaristolactone (33 mg.) in 1 : 1 aqueous dioxan (6 ml.) was mixed with sodium bismuthate (36 mg., containing 80% of $NaBiO_3$) and 80% phosphoric acid (2 ml.) and shaken for 3 hr. (reaction mixture colourless). The suspension was extracted with ether, and the latter dried, and evaporated to yield a pale yellow oil. The oil gave an amorphous 2,4-dinitrophenylhydrazone, but attempts to recrystallise it caused decomposition.

Dehydrogenation Experiments.—(*a*) Semi-solid residues remaining from the preparation of isohexahydroisoaristolactone were refluxed with 20% palladium–charcoal. The violet liquid was slowly distilled, dissolved in cyclohexane, and extracted with 90% phosphoric acid. The acid solution was diluted with water and the azulene re-extracted into cyclohexane for spectroscopic examination. The visible spectrum showed only broad general absorption with no marked maxima; ultraviolet absorption maxima at 244, 279, 289, 306, 333, and 348 mμ. Susz, Pfau, and Plattner [8] give $\lambda_{max.}$ at 290, 308, 336, and 350 mμ and Sörensen and Hougen [8] give $\lambda_{max.}$ 251, 284, 292, 311, 335, 351 mμ for vetivazulene. In oxygen-free 50% sulphuric acid, the azulene showed maxima at 227, 269, and 374 mμ. Chopard-dit-Jean and Heilbronner [8] give $\lambda_{max.}$ for vetivazulene in 50% sulphuric acid at 228, 272, 374 mμ.

(*b*) Crystalline isohexahydroisoaristolactone (400 mg.), dehydrogenated with 20% palladium–charcoal (200 mg.) at 330° for 6 hr., gave no azulenic material, but extraction of the residual mixture with ether gave a brownish oil (30 mg.) which showed well-defined maxima at 228 and 281 mμ and a low-intensity maximum at 312 mμ.

(*c*) Dehydrogenation of oily material (150 mg.) remaining from the preparation of isoaristolactone also gave a violet azulene, $\lambda_{max.}$ 279, 288, 306, 333, and 348 mμ in cyclohexane, and $\lambda_{max.}$ 226, 268, and 374 mμ in 50% sulphuric acid.

(*d*) Hydrogenation was followed by dehydrogenation for oily material (600 mg.) from the preparation of isoaristolactone at 330°. The distillate in hexane deposited colourless crystals (<1 mg.), m. p. 144—145° (block), $\lambda_{max.}$ at 229, 287, and 318 mμ. The azulene, separated as before, had $\lambda_{max.}$ at 280, 289, 306, 333, and 348 mμ in hexane.

TABLE 2.

No.	Compound	No. of double bonds	Reaction time (min.)	Bromine no. (equiv. double bonds)
1	Lupenyl acetate	1	2, 10, 2	1·07, 1·50, 1·55 *
2	Cholesterol	1	2	1·02, 1·09
3	β-Sitosterol	1	10	1·30
4	α-Angelicalactone	1	2, 10	0·77, 0·90
5	Hederagenin Me ester diacetate	1	2, 10	0·65, 0·90
6	Cycloeucalenyl acetate	—	2, 10, 10 (16 hr.)	0·024, 0·076, 0·250 * (1·110)
7	Dihydroaromadendrene	—	2, 10 (16 hr.)	0·03, 0·06 (0·16)
8	Aristolactone (I)	3	2; 10	2·05, 2·35, 2·54, 2·63 *; 2·58, 2·56 *
9	Isoaristolactone (XIII)	3	2, 10 (16 hr.)	2·03, 2·35 (2·59)
10	6,7-Dihydroxyaristolactone (VIII)	2	2, 10	1·90, 2·25
11	Iso-oxoaristaldehyde (IX)	2	2, 10	1·90, 1·98

* Chloroform as solvent.

Tetrahydroisoaristo-6,12-*diol* (XXIV).—Isohexahydroisoaristolactone (470 mg.) in sodium-dried ether (40 ml.) was mixed with lithium aluminium hydride (230 mg.), added portionwise during 15 min. at room temperature, and reaction allowed to proceed for a further 90 min. Excess of reagent was destroyed by dropwise addition of hydrochloric acid and water (10 ml.). Extraction with ether gave colourless needles (340 mg.; from light petroleum–ether) of *tetrahydroisoaristo*-6,12-*diol*, m. p. 106—107° (block), $[\alpha]_D^{20}$ +18·7° (*c* 0·3592 in chloroform) (Found: C, 74·5; H, 12·5. $C_{15}H_{30}O_2$ requires C, 74·35; H, 12·5%). Reaction with benzoyl chloride in pyridine gave the 12-*monobenzoate* as a colourless viscous oil, $[\alpha]_D^{23}$ +1·9° (*c* 2·41 in chloroform) (Found: C, 75·4; H, 10·1. $C_{22}H_{34}O_3$ requires C, 76·2; H, 9·9%).

Determination of Bromine Numbers.—Bromine numbers were determined by the pyridine bromide method for the determination of iodine value of the British Pharmacopoeia 1953, but with modifications of (*a*) sample size (2—10 mg.), (*b*) solvent (carbon tetrachloride or chloroform), and (*c*) reaction time (2 min., 10 min., or 16 hr.). The results are shown in Table 2.

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