Chemistry of the Coprosma *Genus. Part IX.* The Constitution of Asperuloside.*

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Asperuloside, $C_{18}H_{22}O_{11},H_2O$, is shown to be the β-glucoside of the enolic form of a β-ketonic δ-enolic lactone. This structure (XII) accounts for the unusual properties of the compound. Anomalous results in the infra-red spectra are discussed.

ASPERULOSIDE (see Part VIII * for references) has unusual properties, notably that acid hydrolysis produces a clear blue colour, quickly changing to green, followed by the formation of a brownish-black precipitate in a clear supernatant solution. When first prepared, the precipitate dissolves in glacial acetic acid with a green colour and in alkali with a red colour, but when its solution is boiled in air (oxidation) a black, insoluble polymer is formed. Aucubin gives a similar black polymer and is known (Karrer and Schmid, *Helv. Chim. Acta*, 1946, **29**, 525) to be a furan derivative. This similarity and the fact that asperuloside apparently gives the Ehrlich and other reactions typical of furans led Trim and Hill (*Biochem. J.*, 1952, **50**, 310) to suggest a furan structure for asperuloside. However, their furan colour reagents are acidic or potentially acidic so that the colours could be those produced by asperuloside with the acid alone.

Trim and Hill have suggested a formula, $C_{17}H_{24}O_{11}$, for asperuloside but our results with the free compound and its derivatives support the formula of a monohydrate, $C_{18}H_{22}O_{11},H_2O$. It has been extremely difficult to obtain consistent analytical results to determine the molecular formula and especially the water of crystallisation. Analyses of air-dried asperuloside agree with the molecular formula, $C_{18}H_{24}O_{12}$. On heating *in vacuo*, decomposition occurs before drying is complete. The combined results support formulation as a monohydrate [the Karl Fischer method gave variable results (1·9—3·5%), suggesting that the reagent reacted with asperuloside (cf. Mitchell, *Analyt. Chem.*, 1951, **23**, 1069)]. Anhydrous asperuloside also could not be obtained crystalline after azeotropic removal of water.

Asperuloside may be readily characterised as a tetra-acetate (cf. Trim and Hill, *loc. cit.*), tribenzoate (Part VIII), and tri-*p*-nitrobenzoate. Although the formulation as a tetra-acetate is proved by elementary combustion, acetyl values indicated the presence of six acetyl groups. Asperuloside itself contains an acetyl group and the sixth molecule is produced by hydrolytic break-down (see below). The infra-red spectrum of the acetyl derivative contained no hydroxyl bands, indicating that all free hydroxyl groups had been acetylated. Since asperuloside is a glucoside (Juillet, Susplugas, and Massa, *J. Pharm. Chim.*, 1938, **27**, 56; Part VIII), the four acetyl groups must have entered the glucose portion of the molecule. The benzoyl and the *p*-nitrobenzoyl derivative exhibit a hydroxyl band in the infra-red. The acylated derivatives are all acid-labile, giving the same colour changes as asperuloside.

Acid hydrolysis of asperuloside affords glucose as well as the black polymer. Neither colour nor polymer is formed by boiling dilute acid in absence of oxygen. The hydrolysis yields also 1 mol. of carbon dioxide and more than 1 mol. of acetic acid. One mol. of acetic acid results from the hydrolysis of an acetyl group and the further mol. is liberated by hydrolytic break-down (see below). No other acid could be identified after the hydrolysis by paper chromatography by use of Brown's (*Biochem. J.*, 1950, **47**, 598) and Kennedy and Barker's solvent systems (*Analyt. Chem.*, 1951, **23**, 1033).

Before the true nature of the tetra-acetyl derivative was realised it was considered that periodate oxidation might yield valuable information. Asperuloside consumed 2·67 mols. of periodate with the liberation of 1·00 mol. of formic acid (Fig. 1). The glucose portion of the molecule would account for 2 mols. of periodate and 1 mol. of formic acid and the excess of 0·67 mol. of periodate would indicate a further glycol group. As no further

* Part VIII, *J.*, 1954, 3940.

hydroxyl groups are present this represents an anomalous reaction of asperuloside : perhaps the methylene group of the β-ketonic lactone group (see below) is oxidised (cf. Huebner, Ames, and Bubl, *J. Amer. Chem. Soc.*, 1946, **68**, 1621).

Aqueous solutions of asperuloside, when treated with cold sodium hydroxide, carbonate, or hydrogen carbonate solution, more slowly with the weaker alkalis, become yellow and finally reddish-brown, two equivalents of alkali being consumed (Fig. 2). Trim and Hill (*loc. cit.*) observed that 1·3—1·4 mols. of alkali were consumed but that more acid was liberated on standing. In our experiments hydrolysis was extremely rapid—a 0·0075M-solution of asperuloside was completely hydrolysed by 0·1N-sodium hydroxide at 25° in three minutes. Potentiometric titrations showed that asperuloside was neutral, that the two acidic groups set free on hydrolysis were of comparable strength (pK_a ~4·9), and that no phenolic groups were liberated. From these results asperuloside must contain two ester groupings or an anhydride linkage. The very rapid hydrolysis suggested the latter and this appeared to be supported by sodium methoxide titrations. Smith and Bryant (*J. Amer. Chem. Soc.*, 1936, **58**, 2452) have shown that esters undergo methanolysis without uptake of sodium methoxide ($R{\cdot}CO_2R' + NaOMe \longrightarrow R{\cdot}CO_2Me + NaOR'$) while acid

FIG. 1.

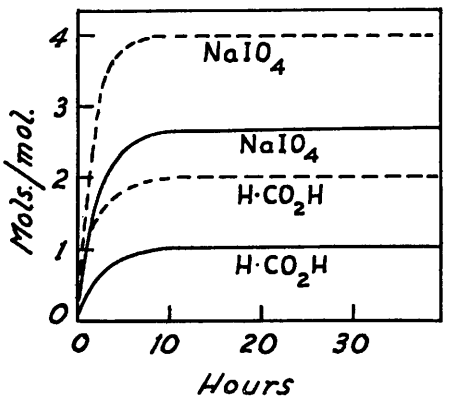
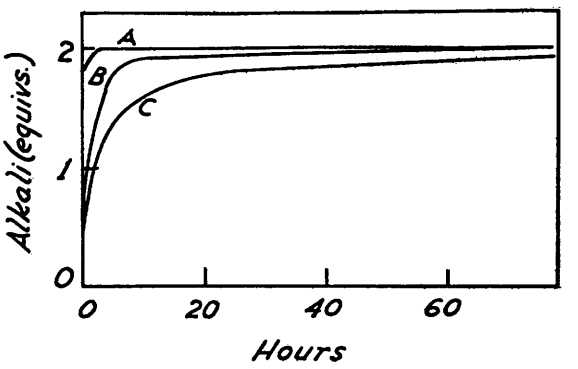


FIG. 2.



anhydrides consume 1 mol. of sodium methoxide ($R{\cdot}CO{\cdot}O{\cdot}CO{\cdot}R' + NaOMe \longrightarrow R{\cdot}CO_2Na + R'{\cdot}CO_2Me$). Both asperuloside and its tetra-acetate consume 1 mol. of sodium methoxide when so titrated. This apparently is a further anomalous reaction since no normal anhydride derivatives could be prepared from asperuloside, and under much milder conditions (Fig. 2) the two acid fragments were liberated at different rates, facts which could not be accommodated by an anhydride group.

Back-titration of hydrolysed solutions of asperuloside, however, gave rather indefinite end-points characteristic of lactones. Further degradation has shown that there is indeed a lactone ring in the molecule and this could explain the anomalous sodium methoxide titrations. Fittig (*Annalen*, 1891, **267**, 191) noted that γ-lactones on treatment with sodium alkoxides gave the corresponding γ-alkoxy-acid. Smith and Bryant (*loc. cit.*) also noted that some lactones behave anomalously, glucono-δ-lactone, for example, consuming 1 mol. of sodium methoxide.

Acetyl values of asperuloside itself were also anomalous, lying between those required for one and two acetyl groups.

The presence of two " ester " groups in asperuloside can be accommodated by the presence of one lactone and one acetyl group and some explanation must be sought for the formation of the other molecule of acetic acid produced on hydrolysis. No crystalline compounds could be isolated after complete or partial hydrolysis, or after methanolysis of asperuloside but acetylation of the hydrolysed product afforded a small yield of tetra-acetylasperuloside. The partial formula, $C_9H_8O(O{\cdot}C_6H_{11}O_5)(OAc)(\cdot O{\cdot}CO\cdot)$, can then be assigned to asperuloside, leaving one oxygen atom to be accounted for.

Asperuloside and its tetra-acetate failed to react with the normal carbonyl reagents and gave no benzylidene derivative. Trim and Hill (*loc. cit.*), however, have shown that asperuloside gives a positive reaction with Duke's test (*Ind. Eng. Chem., Anal.*, 1944, **16**, 110), with a value approximating to one carbonyl group. The presence of a keto-group has been confirmed by the properties of some of its degration products (see below) and is supported by the infra-red spectrum. Asperuloside and its tetra-acetate each exhibit three bands in the carbonyl region, at 1786, 1748, 1701 and 1773, 1748, 1706 cm.$^{-1}$ respectively. The bands at 1701 and 1706 are assigned to the lactone group, those at 1748 to the acetyl group or groups, and those at 1786 and 1773 to the carbonyl group. The inactivity of asperuloside towards carbonyl reagents is probably due to steric hindrance since it will be shown below that the carbonyl group is $\alpha\alpha'$-substituted. The partial formula for asperuloside can now be expanded to $C_8H_8(O{\cdot}C_6H_{11}O_5)(OAc)(\cdot O{\cdot}CO\cdot)(CO)$, derived from a parent hydrocarbon $C_{10}H_{16}$ and thus requiring the presence of three double bonds, three rings, or some combination of the two.

Asperuloside and its tetra-acetate gave a negative test with tetranitromethane and there was only a slow uptake of bromine from the usual solvents. Both were recovered quantitatively after 24 hours' contact with perbenzoic or monoperphthalic acid. Bromine in anhydrous methyl alcohol, however, yielded an acid-stable dibromomethoxide, $C_{18}H_{22}O_{11}(OMe)_2Br_2$. Tetra-acetylasperuloside yielded with the same reagent under slightly more vigorous conditions a monobromo-methoxide, $C_{18}H_{18}O_{11}Ac_4(OMe)Br$, which reacted further with bromine in acetic acid to give a bromo-acetoxy-derivative, $C_{18}H_{18}O_{11}Ac_4(OMe)(OAc)Br_2$. Similarly, tetra-acetylasperuloside with bromine in acetic acid yielded additively a monobromo-acetoxy-derivative, $C_{18}H_{18}O_{11}Ac_4(OAc)Br$. Analogous reactions noted in the literature are usually only side-reactions under the above experimental conditions. One exception is plumieride (Schmid, Bickel, and Meijer, *Helv. Chim. Acta*, 1952, **35**, 415) which resembles asperuloside in giving a polymer on acid hydrolysis and similar anomalous bromination products. The above results indicate the presence of two double bonds of unusual type and therefore one ring besides that of the lactone.

Asperuloside and its tetra-acetate, even in high concentration, exhibit maximum ultra-violet absorption at one point only, 234·5 mμ with ε 6800 and 8300 respectively, this being characteristic of a diene system (Braude, *Ann. Reports*, 1945, **42**, 105) which is confirmed by the fact that asperuloside couples with diazotised *p*-nitroaniline. Neither compound, however, reacted with maleic anhydride or *p*-benzoquinone, indicating a *trans*-diene system. From the position of the maxima in the spectra, Woodward's hypothesis (*J. Amer. Chem. Soc.*, 1942, **64**, 72) requires that the two double bonds shall not be in the same ring. As expected, none of the bromine addition products exhibits a maximum absorption in the ultra-violet. The conjugation of the two double bonds also explains the non-reactivity to per-acids.

The Kuhn–Roth estimation on asperuloside indicates the presence of one *C*-Me group not included in the acetoxy-group, and this completes the functional groups present in the molecule.

Now, the instability of asperuloside to acids is even greater than indicated above: the characteristic colour changes are brought about by boiling N/10,000-hydrochloric acid. Such sensitivity is typical of enolic glucosides (Karrer and Schmid, *Helv. Chim. Acta*, 1946, **29**, 525), suggesting that one of the substituents of the diene system may be the –O–Glucose moiety. This is in line with the effect of such a system on the infra-red spectrum. Conjugated dienes usually absorb between 1639 and 1600 cm.$^{-1}$. The electron-donating property of an oxygen atom directly attached to a diene system will create a greater dipole and so increase the rigidity of the system. This in turn increases the vibrational frequency, and lowers the wave-length and increases the strength of the absorption band. Both asperuloside and its tetra-acetate exhibit an extremely strong band at 1661 cm.$^{-1}$ due, we suggest, to the above causes (it will be seen below that this effect is duplicated).

The band at 814 cm.$^{-1}$ indicates that one of the double bonds is trisubstituted (Sheppard, *Quart. Reviews*, 1952, **6**, 1). The absence of other characteristic bands indicates that both double bonds are trisubstituted or that one is tri- and the other tetra-substituted.

The results of hydrogenation were complex. On hydrogenation asperuloside absorbed 4·6 mol. of hydrogen with palladium–charcoal and slightly larger amounts with platinum catalysts. Tetra-acetylasperuloside absorbed 3·5 mols. with palladium and again slightly more with platinum catalysts. Hydrogenation of asperuloside with palladium catalysts gave 1 mol. acetic acid and a small amount of glucose, but no other crystalline material. Hydrogenolysis of glucose under mild conditions is another characteristic of enolic glucosides (cf. aucubin; Karrer and Schmid, *loc. cit.*). Acetylation of the crude hydrogenated product gave the same product as was obtained by hydrogenation of tetra-acetylasperuloside. Acetic acid was also liberated on hydrogenation of tetra-acetylasperuloside with palladium or platinum catalysts, the major product of the reaction being an acid (A), which gave no colour on hydrolysis with acids. From the analysis of the acid (A), $C_9H_{12}O[O{\cdot}C_6H_7O_5(Ac)_4]{\cdot}CO_2H$, it is apparent that hydrogenolysis of both the acetoxy- and the lactone group has taken place. The infra-red spectrum of this compound contains bands at 1757, 1715, 1706 and a medium band at 1650 cm.$^{-1}$, assigned to acetyl-carbonyl, carboxyl-carbonyl, and carbonyl groups, and a double bond, respectively. The band at 814 cm.$^{-1}$ in asperuloside and its tetra-acetyl derivative disappears on hydrogenation, indicating that the trisubstituted double bond has been reduced. Compound (A) reacted slowly with bromine in methyl alcohol, acetic acid, or chloroform, but no crystalline products could be obtained. Its ultra-violet spectrum exhibited no selective absorption, thus indicating the absence of conjugation of double bonds. No band other than the medium band at 1650 cm.$^{-1}$, characteristic of double bonds, appeared in the infra-red spectrum, suggesting the presence of a tetrasubstituted double bond, in keeping with its resistance to hydrogenation. However, normal tetrasubstituted double bonds, owing to their symmetrical nature, give rise to very weak absorption, if any. The explanation of the anomalous result is that compound (A) is also an enolic glucoside, being hydrolysed by N/1000-hydrochloric acid. The substituent, –O–Glucose, would be expected to increase the dipole of the double bond, and so produce a band of greater intensity than that usually found in a tetrasubstituted double bond.

With diazomethane the acid (A) afforded the methyl ester with the expected properties and infra-red spectrum.

The acid (A) was decarboxylated above its melting point (at 200°) or, better, with a trace of copper in quinoline at 180°. The compound formed, (B), $C_{23}H_{32}O_{11}$, was that expected from a simple loss of carbon dioxide. The infra-red spectrum exhibited the double-bond absorption band of moderate intensity at 1650 cm.$^{-1}$ and only two carbonyl bands (1770 and 1715 cm.$^{-1}$), the carbonyl band of the carboxyl group having disappeared. Acid hydrolysis of the product (B) gave a steam-volatile diketone (C), $C_9H_{14}O_2$, proving conclusively that the parent compounds are enolic glycosides. The diketone, obtained directly or by regeneration from its 2 : 4-dinitrophenylhydrazone, gave a positive iodoform test, indicating the presence of a COMe group. Since the compound (B) failed to give the test before hydrolysis it must contain the grouping, Glucosyl·O·CMe:C$\lessdot^{C}_{C}$, including the tetrasubstituted double bond, to agree with the infra-red spectrum.

Compound (A) on acid hydrolysis also afforded the diketone (C), with loss of carbon dioxide. This contrasts with the more violent conditions required for thermal decarboxylation, indicating that (A) is a potential β-ketonic acid. There is then only one way of placing the carboxyl group in a non-conjugated position to the double bond as required by the ultra-violet spectrum, namely, (I).

(I) $C(O{\cdot}G)=C-C=CH_2{\cdot}CO_2H$ (G = glucosyl) ; $C(O{\cdot}G)=C-C(R,H)=C-CH_2{\cdot}CO_2H$ (II)

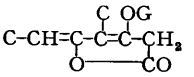
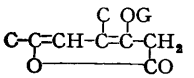
In apseruloside there is a further trisubstituted double bond, conjugated with that in (I), so that asperuloside must contain the partial skeleton (II).

Trim and Hill (*loc. cit.*) observed various colour reactions when asperuloside was treated with a variety of amines. These have been confirmed in that it has been found that

asperuloside reacts with primary aliphatic or aromatic amines to give yellow solutions, rapidly changing through orange to red and finally yielding deep blue to greenish-blue water-soluble pigments, from which, however, no crystalline product could be isolated. The reaction is not concerned with the keto-group (as suggested by Trim and Hill) since the hydrogenated product (A) failed to give the colours with primary amines. These colour reactions, we suggest, are due to the presence of an enolic lactone grouping, since Walton (*J.*, 1940, 438) observed similar colour reactions with compounds of this type.

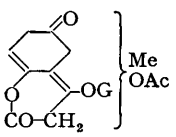
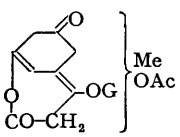
An aqueous solution of asperuloside, originally neutral, becomes acid to litmus when boiled for ½ hour. No volatile acid could be distilled, showing that only the lactone group was involved. This property is also characteristic of enolic lactones, since enolic lactones in boiling water afford some keto-acid (Kuehl, Linstead, and Orkin, *J.*, 1950, 2213) while normal esters, lactones, or even αβ-unsaturated lactones do not react as rapidly or as readily.

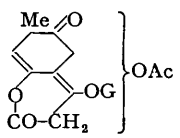
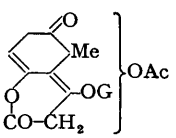
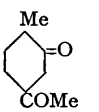
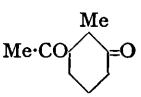
Hydrogenation of asperuloside or its tetra-acetate yielded the corresponding deoxy-acid, another feature of enolic lactones (Jacobs and Scott, *J. Biol. Chem.*, 1930, **87**, 60). It appears, therefore, that asperuloside contains the partial skeleton (III) or, much less probably, (IV). On to this structure must be fitted a carbon ring and a carbonyl group,

(III)   (IV)

placed in a position non-conjugated with the double bonds (ultra-violet spectrum), together with a methyl and an acetoxy-substituent. There are thus only two partial structures, *viz.*, (V) and (VI). The latter doubly contravenes Bredt's rule (Fawsett, *Chem. Reviews*, 1950, **47**, 219) and may be eliminated. Thus asperuloside has one of the partial structures (VII) or (VIII).

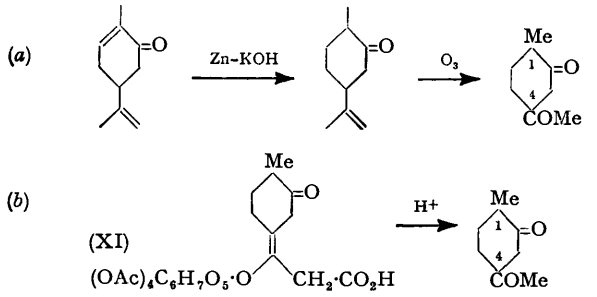
On these formulations, the diketone (C), formed on hydrolysis of the hydrogenated derivatives, must be (IX) or (X). On phytochemical grounds, the former, related to *p*-menthane, is to be preferred. This ketone has been prepared by Wallach and Schrader

(V)   (VI)

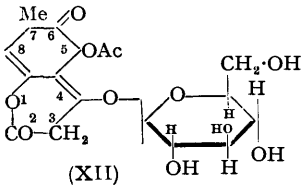
 (VII)  (VIII)  (IX)  (X)

(*Annalen*, 1894, **279**, 377) as a minor oxidation product of dihydrocarvone. It has now been prepared by the ozonolysis of dihydrocarvone, and its bis-2 : 4-dinitrophenylhydrazone has been prepared. The melting point, 242°, is different from that, 225°, of the diketone (C) and depresses its melting point. The infra-red spectra of the two compounds, however, were practically identical, exactly in the intensities and positions of the absorption bands in the valency region (2—7 μ) and only slightly different in the higher regions; the main difference was a weak band at 857 cm.$^{-1}$ for the product from dihydrocarvone which did not appear in the other. However, neither product is optically pure. That obtained from dihydrocarvone by reaction (*a*) is asymmetric at $C_{(4)}$, but racemic at $C_{(1)}$. And if our assumption on the structure of the diketone (C) is correct the hydrogenated tetra-acetyl-asperuloside must have formula (XI), leading by reaction (*b*) to the diketone asymmetric at $C_{(1)}$ and racemic at $C_{(4)}$. This difference would account for the physical differences observed. Attempts to racemise either ketone by acid or alkali led to decomposed products. The structure was confirmed by oxidation of hydrogenated tetra-acetylasperuloside (A) under vigorous conditions with alkaline permanganate and nitric acid to α-methyl-

glutaric acid, which could not be obtained from any isomeric structure for the diketone. The rotation of this acid could not be obtained owing to the small amount isolated.

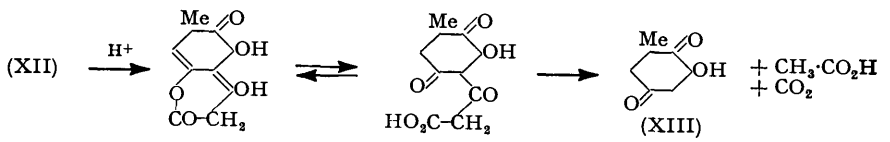


The final problem was the position of the acetoxy-group. If it were attached to a tertiary carbon atom, this would explain its ready hydrogenolysis (cf. andrographolide; Kleipool and Kostermans, *Rec. Trav. chim.*, 1951, **70**, 1085). However, tertiary acetates are usually cleaved by aluminium amalgam at room temperature (Gorter, *ibid.*, 1914, **33**, 239) and asperuloside and its tetra-acetate were unaffected by this reagent even under more vigorous conditions (boiling aqueous-dioxan). A potential ketol group, however, was disclosed by the following experiments. When asperuloside was added to Fehling's solution, the colour changed first to green and then slowly to blue, different from the original colour of the reagent. Warming with *excess* of the reagent produced a copious precipitate of cuprous oxide. Earlier workers reported that asperuloside does not reduce Fehling's solution. The colour changes are apparently due to chelation of copper after hydrolysis of the asperuloside. Nickel and iron form chelate compounds with hydrolysed asperuloside so stable that the metal hydroxides are not precipitated by alkali. Unhydrolysed asperuloside does not form chelate compounds. Chelation, we suggest, is due to the presence of an α-ketol group. Asperuloside readily reduced Tollens's reagent and ammoniacal silver nitrate, as observed by earlier workers, possibly owing to the presence of the α-ketol group liberated on hydrolysis or the γδ-unsaturated lactone group (cf. Kuehl *et al.*, *loc. cit.*), but there was no action with Rigby's bismuth trioxide reagent (*J.*, 1951, 793). Hydrolysed asperuloside, however, gave a positive test with bismuth trioxide, confirming the presence of an α-ketol group in this material and a potential α-ketol group in asperuloside itself. Asperuloside, therefore, must have the structure (XII).



(XII)

An alternative structure with the acetoxy-group on $C_{(8)}$ does not contain a trisubstituted double bond as required by the infra-red spectrum, nor does it explain the ready hydrogenolysis of the acetoxy-groups. The above formula is supported by the infra-red data and the observations of Adkins (see Gilman, " Organic Chemistry : An Advanced Treatise," John Wiley & Sons, Inc., New York, Vol. I, p. 820) that C–O bonds are most readily hydrogenolysed when adjacent to an electrophilic group (aromatic, carbonyl, alkoxycarbonyl, etc.). Two such groupings on either side of the acetoxy-group in asperuloside thus leads to the ready hydrogenolysis observed.



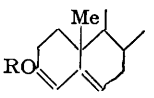
Final support for the structure of asperuloside came from a study of the black polymer formed on oxidative hydrolysis. The first product, in its free acid form, is a βδ-diketo-acid which could hydrolyse in various ways, but all these would give the same intermediate (XIII). This would explain the production of carbon dioxide and the 2 mols. of acetic

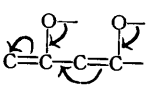
acid and thus the anomalous acetyl values (p. 4182). The intermediate compound (XIII), being a β-hydroxy-ketone, would readily lose water to form toluquinol; oxidation to toluquinone could then occur, with the formation of the quinhydrone, which is known to polymerise. It is also evident why oxidation is necessary in addition to hydrolysis.

On this basis the black precipitate should be polymerised toluquinhydrone. The formula, $(C_7H_7O_2)_n$, agreed with this while the infra-red spectra of the polymer and that synthesised from authentic toluquinhydrone were practically identical. When freshly prepared, polymerised toluquinhydrone is soluble in glacial acetic acid and alkalis, yielding green and red colours respectively, bleached by reducing agents (hydrogen peroxide, sulphur dioxide, zinc, etc.), and the polymer from asperuloside behaves similarly. Finally, a black polymer is obtained, insoluble in acids, alkalis, organic solvents, and even in concentrated sulphuric acid again similar to the final product from asperuloside. Polymerisation, however, occurs apparently by a somewhat different mechanism since asperuloside is polymerised by boiling 2N-hydrochloric acid in five minutes whereas toluquinhydrone requires several hours for complete reaction.

In agreement with the above mechanism, boiling 5% anhydrous methanolic hydrogen chloride does not bring about the usual colour changes. Methanolysis of enolic lactones to the corresponding keto-esters is extremely rapid (Kuehl *et al.*, *loc. cit.*). Thus, asperuloside gives a β-keto-ester which would not decompose as does the free β-keto-acid obtained on aqueous hydrolysis.

Two further observations on the spectra of asperuloside can be made. Woodward (*loc. cit.*) correlated the position of the absorption bands of diene systems with substitution by alkyl groups. Little difference apparently occurs when one substituent is an oxygen atom, *e.g.*, progesterone enol acetate and testosterone diacetate; with the system (XIV), both exhibit maximum absorption at 240 mμ, compared with a theoretical value of 242 mμ (Woodward). When two substituent oxygen atoms are present, however, as in the pentasubstituted diene system of asperuloside, a marked change occurs. The absorption maximum of asperuloside lies at 234·5 mμ instead of 247 mμ required for a pentasubstituted diene. Again, in the infra-red spectra, two cumulative electron-donating oxygen atoms in the system (XV) give rise to a greater dipole. This is reflected in increased rigidity, leading to a higher vibrational frequency, *i.e.*, a lower wave-length and a greater intensity of the absorption band. Rosenkrantz and Gut (*Helv. Chim. Acta*, 1953, **36**, 1000) recently made similar observations on the increased intensity of the double-bond absorption of enolic lactones and esters.

(XIV) 



(XV)

On this evidence a different interpretation may be placed on the results obtained by Schmid, Bickel, and Meijer (*loc. cit.*) on plumieride, and we suggest that this substance also contains a diene system with oxygen substituents. The ultra-violet spectrum exhibits only one absorption peak at *ca.* 217 mμ, characteristic of a diene system, and not, as suggested by the Swiss workers, of an αβ-unsaturated carbonyl system, where there are usually two absorption bands, the intense *K*-band and the less intense *R*-band at a higher wave-length (cf. Evans and Gillam, *J.*, 1941, 815). Woodward (*loc. cit.*) has shown that substitution of a diene system produces an absorption band at a higher wave-length than that (217 mμ) of butadiene itself. We have shown, however, that substitution with an oxygen atom produces a bathochromic effect. The position of the absorption maximum of plumieride, *ca.* 217 mμ, could be the result of the cancellation of the hypsochromic effect of alkyl substitution on the diene system by the bathochromic effect of oxygen substitution. The relatively strong band at 6·13—6·14 μ (1629—1631 cm.$^{-1}$) in the infra-red spectrum of plumieride could be that of a diene system with oxygen substitution, for reasons advanced for asperuloside itself. There are other reactions also of plumieride reminiscent of the peculiar properties of asperuloside due to the presence of the oxygen substituted diene system.

The unusual constitution of asperuloside might have been reflected in some unusual

physiological effect. We are greatly indebted to Dr. W. F. Short of Messrs. Boots Pure Drug Co. Ltd., for a comprehensive survey of its pharmacological and bacteriological properties, which, however, disclosed no outstanding activity.

Experimental

Analyses are by Drs. T. S. Ma and A. D. Campbell, Otago Univeristy. M. p.s were carried out in Mason's apparatus.

Extraction of Asperuloside.—Finely ground, air-dried bark of *Coprosma robusta*, collected at Mangere, was extracted continuously with acetone until a test portion of the bark on boiling with dilute hydrochloric acid no longer gave a black precipitate. After concentration, the acetone extract was seeded with asperuloside and set aside in the refrigerator. The crystalline mass was then filtered off, washed with ligroin, crystallised from alcohol and then water and finally repeatedly from alcohol to constant m. p. 131·5—132° (yield, 1·4—1·5%). $[\alpha]_D^{18}$ was −200·4° (*l*, 1; *c*, 1·4 in H_2O). The following analytical results were obtained on samples of the same material: Found, on air-dried material: C, 50·0, 50·2; H, 5·8, 5·9; Ac, 16·6, 15·1, 13·9; *C*-Me, 5·7. Calc. for $C_{18}H_{22}O_{11},H_2O$: C, 50·0; H, 5·6; 1Ac, 10·0; 1*C*-Me, 3·6. Found, in material dried to constant weight over P_2O_5 *in vacuo* at room temperature: C, 50·9, 50·7; H, 5·6, 5·6; loss, 0·93, 0·95. Found, after crushing the crystals and drying them to constant weight over P_2O_5 *in vacuo* at room temperature: C, 50·8, 50·8; H, 5·3, 5·5; loss, 1·70, 1·67%. Found, in material dried to constant weight over P_2O_5 *in vacuo* at 78°: C, 51·3, 51·1; H, 5·6, 5·5; loss, 2·43, 2·27. Calc. for $C_{18}H_{22}O_{11}$: C, 52·2; H, 5·3; loss, 4·17%. Samples dried at 100° gradually melted to a brownish-black substance with loss of a crystalline sublimate and gave inconsistent analytical figures. The loss of weight in such experiments varied between 2·75 and 4·3%.

The Karl Fischer reagent, prepared according to Almy, Griffin, and Wilcox (*Ind. Eng. Chem., Anal.*, 1940, **12**, 392), was standardised against chloral hydrate (visual end-points). Terpin hydrate gave values of 9·58 and 9·42% for the water content (calc., 9·48%). Asperuloside gave 2·58, 3·94, and 1·93% (calc. 4·17%).

The molecular weight was determined by Signer's isopiestic method (*Annalen*, 1930, **478**, 246) as modified by Clark (*Ind. Eng. Chem., Anal.*, 1941, **13**, 820). Phenacetin in methanol, with azobenzene as standard, gave *M*, 173 (calc., 179). Asperuloside, under similar conditions, gave *M*, 463 (calc., 432), apparently owing to dissociation of the water of crystallisation. Hérissey (*Compt. rend.*, 1925, **180**, 1695) obtained *M*, 410 (cryoscopic in H_2O) in good agreement with the calculated value of 414.

The ultra-violet absorption spectrum, measured in *ca.* M/5000-alcoholic solution, exhibited a maximum at 234·5 mμ (log ε 3·83). The infra-red spectra of asperuloside and its derivatives were measured in Nujol mulls. Max. for asperuloside appeared at 3497w, 3300m, 3165m, 1786m, 1748s, 1701s, 1661s, 1534w, 1508w, 1330w, 1282s, 1217m, 1185m, 1081s, 1059s, 1025s, 990s, 754m, 913m, 862m, 815m, 765w, 745m, and 727w cm.$^{-1}$ (s = strong, m = medium, w = weak).

Acid Hydrolysis of Asperuloside.—(*a*) Asperuloside (1·573 g.) in water (150 c.c.) and concentrated sulphuric acid (5 c.c.) was steam-distilled until no further acid appeared in the distillate. Neutralisation of the distillate required 42·93 c.c. of 0·1N-sodium hydroxide (calc. for 1 mol. of acid, 36·41 c.c.). Evaporation of the neutralised distillate yielded a brown solid (360 mg.). This material (200 mg.) was dissolved in water (20 c.c.), acidified to litmus with hydrochloric acid, and extracted continuously with ether for 80 hr. On attempted microfractionation of the extremely pungent, brown oil so obtained, extensive decomposition occurred and no organic material could be recovered from the distillate. Sublimation of the remainder, however, at 60°/3 mm. afforded a very small quantity of needle-shaped crystals, micro-m. p. 111—112°, undepressed by the sublimate formed on drying asperuloside at 100°. There was insufficient for further investigation.

The brown solid (50 mg.) was dissolved in water (1 c.c.) and made just acid to phenolphthalein with hydrochloric acid. A cold saturated solution of *S*-benzylthiouronium chloride in alcohol (2 c.c.) was added, and the mixture boiled and allowed to cool. Repeated crystallisation of the product from alcohol afforded rectangular plates, m. p. and mixed m. p. with *S*-benzylthiuronium acetate, 137°. *p*-Nitrobenzyl acetate, m. p. and mixed m. p. 78°, was obtained by similar manipulation.

(*b*) A solution of asperuloside (0·1262 g.) in water (25 c.c.) was freed from dissolved carbon dioxide by bubbling nitrogen through the boiling solution. Concentrated hydrochloric acid (2 c.c.) was added, the boiling and passage of nitrogen was continued for 2 hr. and the issuing

gases were passed through Peligot tubes containing barium hydroxide solution. Barium carbonate (0·0363 g., 0·63 mol.) was collected.

(*c*) The black *polymer* formed on hydrolysis (Hérissey's "asperuligenol," *loc. cit.*), was prepared by boiling asperuloside with dust-free 2N-hydrochloric acid for several hours. The material was washed with water and organic solvents and dried [Found: C, 68·5; H, 5·7. $(C_7H_7O_2)_n$ requires C, 68·3; H, 5·7%].

Hydrolysis of Asperuloside.—0·005M-Solutions of asperuloside were treated with 0·1N- and 0·025N-sodium hydroxide and 0·069N-sodium carbonate solutions at 25° (thermostat), and aliquot portions titrated at intervals. The results are illustrated in Fig. 2.

A solution of asperuloside (108 mg.) in absolute methanol (10 c.c.) and N-alcoholic sodium hydroxide (0·5 c.c.; 2·2 equiv.) was kept at room temperature for 24 hr., during which the solution had become deep brown. Passage of the solution through an ion-exchange column (Amberlite IR-4B) removed both the metal-ions and the colour. Concentration of the filtrate and methanol washings afforded a pale green, viscous gum, decolorised by treatment of an acetone solution with charcoal. The solid obtained on evaporation, however, was extremely hygroscopic and resisted attempts to crystallise it and to form acyl derivatives. Treatment of a small quantity (25 mg.), however, with acetic anhydride (0·75 c.c.) and pyridine (1·5 c.c.) in a sealed tube at 100° for 2 hr. afforded, after crystallisation, colourless plates of tetra-acetyl-asperuloside, m. p. and mixed m. p. 154°.

Periodate Oxidation of Asperuloside.—0·3409M-Sodium metaperiodate (20 c.c., 16 mols.) was added to asperuloside (0·4385 g.) in water (460 c.c.). The mixture was made up to 500 c.c. with water. Temperatures throughout were 25° (thermostat). The amount of periodate consumed and the formic acid produced were determined as described by Briggs and Vining (*J.*, 1953, 2809). The results are illustrated in Fig. 1.

Tetra-acetylasperuloside.—Prepared as recorded in Part VIII (*loc. cit.*) this had m. p. 154·5—155°. A 92% yield, however, was obtained by reaction at room temperature for 2 days [Found, after drying at 100°: C, 53·9, 53·6; H, 5·5, 5·0; Ac, 41·2, 37·3, 37·6%; *M*, isopiestic in (*a*) methyl formate, 573, (*b*) methyl acetate, 576. Calc. for $C_{26}H_{30}O_{15}$: C, 53·6; H, 5·2; 5Ac, 36·9%; *M*, 582·5]. $[\alpha]_D^{17}$ was −128·6° (*l*, 1; *c*, 0·65 in EtOH). The infra-red spectrum exhibited peaks at 1773s, 1748s, 1706m, 1661s, 1253s, 1232s, 1209s, 1771s, 1121m, 1066s, 1037s, 987s, 962s, 912m, 858m, 833w, 813m, 753w, and 746w cm.$^{-1}$. The ultra-violet spectrum, measured in *ca.* M/8500-alcoholic solution, exhibited a maximum at 234·5 mμ (log ε 3·92).

Tribenzoylasperuloside.—This derivative (see Part VIII; m. p. 168—168·5°) had a strong absorption band at 3356 cm.$^{-1}$, assigned to hydroxyl.

Tri-p-nitrobenzoylasperuloside.—Asperuloside (200 mg.), *p*-nitrobenzoyl chloride (1·76 g.), and pyridine (2·5 c.c.) were heated under reflux for 1 hr. The cooled mixture was stirred into ice-water (100 c.c.) and allowed to stand. The *ester* (85%), after crystallisation to constant m. p. from ethyl acetate–alcohol (2:3), formed colourless needles, m. p. 222—222·5° (Found, in material dried at 110°: C, 54·7, 54·9; H, 3·9, 3·4; N, 5·1, 5·2. $C_{39}H_{31}O_{20}N_3$ requires C, 54·4; H, 3·6; N, 4·9%). The infra-red spectrum showed a maximum at 3356 cm.$^{-1}$, assigned to hydroxyl.

Anhydride Titrations.—These were carried out according to Smith and Bryant (*J. Amer. Chem. Soc.*, 1936, **58**, 2452), the sample being dissolved in 4 c.c. of *ca.* 0·06N-sodium methoxide in a stoppered flask and then heated for 1 hr. at 50°. After cooling, the sample solution and a blank, similarly treated, were titrated with 0·0134N-hydrogen chloride in anhydrous methanol to an end-point of pH 9·5 (Cambridge potentiometer) [Found: Mols. of sodium methoxide consumed, (*a*) asperuloside, 0·87 (½ hr. heating only), 1·08 (*b*) tetra-acetylasperuloside, 0·93, 1·10].

Asperuloside Dibromomethoxide.—A solution of asperuloside (100 mg.) in methanol (6 c.c.) was treated with bromine (300 mg.), warmed at 50° for 15 min., cooled, and poured into ether (50 c.c.). The green gum so obtained solidified on trituration with ligroin and, after repeated crystallisation from 15% methanol, gave the *dibromo-methoxide* as colourless, rectangular plates, m. p. 178° (decomp.), $[\alpha]_D^{17}$ −23·6° (*l*, 1; *c*, 0·55 in EtOH (Found, in material dried at room temp.: C, 37·8, 37·4, 38·0; H, 4·7, 4·5, 4·6; Br, 25·4, 25·2; OMe, 9·2. $C_{20}H_{28}O_{13}Br_2$ requires C, 37·8; H, 4·5; Br, 25·1; 2OMe, 9·7%). The infra-red spectrum exhibited peaks at 3497w, 3300m, 1773m, 1745s, 1704s, 1508w, 1337w, 1277m, 1217m, 1189m, 1083s, 1063s, 1026s, 1002s, 966s, 924w, 903w, 856m, 810w, 775w, and 728w cm.$^{-1}$.

Acetylation (pyridine–acetic anhydride, 100°, 4 hr.) afforded the *tetra-acetyl* derivative, colourless, hexagonal plates (from alcohol), m. p. 152·5—153° (Found, in material dried at room temp.: C, 41·9; H, 4·4; Br, 19·8; OMe, 6·4; Ac, 34·5, 32·2. $C_{28}H_{36}O_{17}Br_2$ requires C, 41·8; H, 4·5; Br, 19·9; 2OMe, 7·7; 5Ac, 26·7%).

Tetra-acetylasperuloside Monobromoacetoxylate.—Bromine (750 mg.) in glacial acetic acid

(2·5 c.c.) was added dropwise to tetra-acetylasperuloside (100 mg.) in the same solvent (1 c.c.). After 1 hr. at room temp. the solution was heated at 100° for 15 min., cooled, and stirred into ice-water (50 c.c.). The precipitated *derivative* crystallised from alcohol in colourless plates, m. p. 183·5—184° (Found, in material dried at 100°: C, 46·8; H, 4·8; Br, 11·0; Ac, 36·6. $C_{28}H_{33}O_{17}Br$ requires C, 46·6; H, 4·6; Br, 11·1; 6Ac, 35·8%). Bands in the infra-red spectrum appeared at 1776s, 1745s, 1706w, 1647w, 1368m, 1340w, 1319w, 1240m, 1229s, 1202s, 1163w, 1143w, 1105m, 1049s, 1014m, 981s, 933m, 906m, 867m, 809w, and 779w cm.$^{-1}$.

Tetra-acetylasperuloside Bromomethoxide.—A solution of tetra-acetylasperuloside (200 mg.) and bromine (150 mg.) in methanol (5 c.c.) and pyridine (1 c.c.) was set aside overnight and then evaporated *in vacuo* at 100°. Crystallisation of the gummy residue from 85% alcohol afforded silky needles of the *product*, m. p. 154·5—155° (decomp.) (Found, in material dried at 100°: C, 46·6; H, 4·7; Br, 11·6; OMe, 4·7. $C_{27}H_{33}O_{16}Br$ requires C, 46·8; H, 4·8; Br, 11·5; 1OMe, 4·5%). Bands in the infra-red spectrum appeared at 1773s, 1751s, 1715m, 1647w, 1486m, 1451w, 1374w, 1342w, 1319m, 1309m, 1258s, 1227s, 1215s, 1152m, 1136w, 1099m, 1079m, 1068m, 1044s, 1009m, 971s, 948m, 927m, 910w, 883w, 862w, and 800w cm.$^{-1}$.

Tetra-acetylbromodihydromethoxyasperuloside Bromoacetoxylate.—A solution of the foregoing compound (50 mg.) in glacial acetic acid (1 c.c.) was treated with bromine in the same solvent until decolorisation ceased. The *product* obtained by pouring the mixture into water crystallised from 90% alcohol in large prisms, m. p. 190·5—191° (Found, in material dried at 100°: C, 41·9; H, 4·7; Br, 19·1; Ac, 34·9. $C_{29}H_{36}O_{18}Br_2$ requires C, 41·8; H, 4·4; Br, 19·2; 6Ac, 31·1%).

Hydrogenation of Asperuloside.—Asperuloside (1 g.) in alcohol (120 c.c.) was hydrogenated with a palladium–charcoal catalyst at 45 lb. and room temp. for 14 hr. After filtration, the solution was distilled *in vacuo*. The acid distillate required 20·4 c.c. of 0·1N-sodium hydroxide for neutralisation (0·89 equiv.), and from the neutralised solution the *S*-benzylthiuronium salt was prepared (m. p. and mixed m. p. with *S*-benzylthiuronium acetate, 137°). The gummy residue from the distillation solidified on trituration with light petroleum (b. p. 40—60°). After being washed with alcohol–benzene (2 : 3) it crystallised from 90% alcohol in clusters of rectangular plates, m. p. 152° (23 mg.) (Found, in material dried at room temp.: C, 36·9, 36·7; H, 7·2, 7·0. Calc. for $C_6H_{12}O_6,H_2O$: C, 36·4; H, 7·1%). The material was proved to be glucose by paper chromatography (control, glucose), with butanol–pyridine–water (3 : 1 : 1) (cf. Hough, Jones, and Wadman, *J.*, 1950, 1702). The spots were developed for 30 hr. and sprayed with aniline hydrogen phthalate (Partridge, *Nature*, 1949, **164**, 443).

In a similar experiment, the gummy residue from the distillation was acetylated with acetic anhydride in pyridine at 100° for 4 hr. and poured into water. The oil so obtained was decolorised with charcoal in alcohol and again poured into water. The colourless gum solidified on trituration with hot 20% alcohol, from which repeated crystallisation from 60% alcohol yielded the *acid A* as needles, m. p. 173—173·5°, undepressed on admixture with hydrogenated tetra-acetylasperuloside (see below) (Found, in material dried at 100°: C, 54·8; H, 5·9; Ac, 39·8. $C_{24}H_{32}O_{13}$ requires C, 54·6; H, 6·1; 4Ac, 32·4%).

Hydrogenation of Tetra-acetylasperuloside.—Tetra-acetylasperuloside (1 g.) in alcohol (150 c.c.) was hydrogenated with a palladium–charcoal catalyst (100 mg.) or, better, with platinum oxide (100 mg.) at 45 lb. and room temp. for 24 hr. After filtration, the solution was distilled *in vacuo*, yielding a distillate containing 0·84 equiv. of acetic acid. Most of the clear gum remaining after distillation dissolved in sodium hydrogen carbonate solution and yielded a pasty solid on acidification. Repeated crystallisation from 55% methanol afforded colourless, silky needles of β-(4-*methyl*-3-*oxo*cyclo*hexylidene*)-β-*tetra*-O-*acetylglucopyranosyloxypropionic acid* (compound A) (43%), m. p. 185—185·5°, $[\alpha]_D^{17}$ −84·1° (*l*, 1; *c*, 1·2 in EtOH) (Found, in material dried at 100°: C, 54·7; H, 6·1; Ac, 32·6, 33·7. $C_{24}H_{32}O_{13}$ requires C, 54·6; H, 6·1; 4Ac, 32·4%). The equiv. weight (521, 516) was determined by microtitration to phenol-red of solutions in 50% alcohol with standard alkali (calc. : equiv., 528). Bands in the infra-red spectrum appeared at 3226m, 1757s, 1715s, 1706s, 1645m, 1441w, 1366m, 1285s, 1241s, 1217s, 1183s, 1164m, 1119m, 1100m, 1081m, 1062s, 1035s, 966s, 923m, 902m, 855m, 809w, 755w, and 729w cm.$^{-1}$.

The *methyl ester* of this acid was prepared by diazomethane in ether–methanol. The product, after trituration with sodium hydrogen carbonate solution, crystallised from 65% methanol in silky needles, m. p. 109·5—110° (83%) (Found, in material dried at 100°: C, 55·5; H, 6·4; OMe, 5·7; Ac, 33·4. $C_{25}H_{34}O_{13}$ requires C, 55·4; H, 6·3; 1OMe, 5·7; 4Ac, 31·7%). Bands in the infra-red spectrum appeared at 1767s, 1715s, 1706s, 1642m, 1453w, 1374w, 1285m, 1232m, 1220m, 1185s, 1164w, 1119w, 1096w, 1080w, 1043m, 1017w, 971m, 956m, 919m, 904s, 860m, 790m, 768m, and 725w cm.$^{-1}$.

Hydrolysis of Compound A.—The acid (183·2 mg.) was heated with 2N-hydrochloric acid at 100°. Evolved carbon dioxide was removed in nitrogen and absorbed in barium hydroxide solution. Excess of barium hydroxide was titrated with standard hydrochloric acid to phenolphthalein, and then the barium carbonate to methyl-orange (Found : CO_2, 0·87 mol.).

Decarboxylation of Compound A.—When the acid (150 mg.) was heated in quinoline (2 c.c.) with copper carbonate (*ca.* 1 mg.) gas was evolved at 190—205°. After 2 hr. at this temperature, the mixture was heated with acetic anhydride (1 c.c.) for 2 hr. at 100°, cooled and poured into 2N-hydrochloric acid (100 c.c.). The precipitated tar was washed with dilute acid and water, decolorised with charcoal in alcohol, and crystallised from 50% methanol, to yield long, rectangular plates of 2-*methyl*-5-1′-*tetra*-O-*acetylglucopyranosyloxoethyl*cyclo*hexanone*, m. p. 167—167·5° (78%) (Found, in material dried at 100° : C, 55·0, 55·1; H, 6·5, 6·8; Ac, 30·7, 33·0. $C_{23}H_{32}O_{11},H_2O$ requires C, 55·0; H, 6·8; 4Ac, 34·2%). The material, dried at 100°, still gave a positive test with the Karl Fischer reagent. An examination of a restricted portion of the infra-red spectrum revealed peaks at 1770s, 1715s, 1650m, 1441w, and 1377w cm.$^{-1}$.

Hydrolysis of the acid A or its decarboxylated product with boiling 2N-hydrochloric acid afforded a water-soluble ketone, isolated as its *bis*-2 : 4-*dinitrophenylhydrazone* which crystallised from ethyl acetate or nitrobenzene in clusters of orange-red needles, m. p. 224·5—225° (Found, in material dried at 100° : C, 49·1; H, 4·1; N, 20·5, 21·0. $C_{21}H_{22}O_8N_8$ requires C, 49·0; H, 4·3; N, 21·8%). An aqueous solution of the diketone, obtained by hydrolysis of the 2 : 4-dinitrophenylhydrazone with 2N-hydrochloric acid followed by steam-distillation, gave a positive iodoform test (m. p. and mixed m. p. 118°).

5-*Acetyl*-2-*methyl*cyclo*hexanone.*—Dihydrocarvone was prepared from carvone (10 g.) according to Wallach and Schrader (*Annalen*, 1894, **277**, 377) by heating it under reflux with zinc dust (25 g.) and potassium hydroxide (14 g.) in aqueous alcohol (175 c.c.; 1 : 2) for 5 hr. Most of the alcohol was distilled off on a water-bath and the residue saturated with salt and distilled in steam. The distillate (1 l.) was extracted with ether (3 × 150 c.c.), the ethereal solution dried, and the solvent removed. The crude material was purified *via* the bisulphite compound from which dihydrocarvone (6·5 g.) was liberated by treatment with 2N-sodium hydroxide. The oxime, prepared in alcoholic pyridine at 100° for 3 hr., crystallised from 40% alcohol in needles, m. p. 88—89° (cf. Wallach and Schrader, *loc. cit.*).

Dihydrocarvone (5 g.) was ozonised in the usual way in ethyl acetate, and the ozonide decomposed by hydrogenation with a palladium–charcoal catalyst. The oil recovered was fractionated under reduced pressure, yielding fractions, (*a*) b. p. 25—120°/4 mm. (1·6 g.) and (*b*) b. p. 120—128°/4 mm. (1·3 g.). Fraction (*b*) gave a semicarbazone, needles (from 60% alcohol), m. p. 207—208° (84%), and an oxime, prisms (from alcohol), m. p. 195—196° (72%). Fraction (*a*) contained the same diketone, giving the same semicarbazone (48%). Wallach and Schrader (*loc. cit.*) record m. p.s 203—204° and 195° for these derivatives.

The bis-2 : 4-dinitrophenylhydrazone from fraction (*b*) crystallised from ethyl acetate or nitrobenzene in clusters of orange needles, m. p. 241·5—242° (72%). The infra-red spectra of this and the corresponding compound from the degradation of asperuloside are recorded below. The bands were practically identical, discrepancies in the asperuloside derivative being recorded in parentheses : 724·6w (725·0), 738·5w (740·2), 761·2w (760·1), 835·8m, 856w (—), 920·0m, 946·1w, 971·8w, 1047w (1046), 1074w (1703), 1139s, 1220m, 1272s, 1312s, 1333s, 1374w, 1401w, 1420w, 1437w, 1462w, 1475w, 1511m, 1592w, 1656m, 1689w, 1748w, 1786w, 1812w, and 1845w cm.$^{-1}$.

Oxidation of Compound A.—Potassium permanganate solution (2%) was added to the above acid (709 mg.) in 2N-sodium hydroxide (200 c.c.) until a pink colour remained after ½ hr. The precipitated manganese dioxide was dissolved by passage of sulphur dioxide, and the solution evaporated to dryness. Concentrated nitric acid was then added and the solution again evaporated to dryness. The residue was exhaustively extracted with benzene, and the acids so recovered were sublimed *in vacuo*. The fraction subliming at 190—210°/12 mm. crystallised from benzene (yield, 24 mg.) and finally yielded prisms, m. p. 78—79° (Found, in material dried at room temp. : C, 49·3; H, 7·1. Calc. for $C_6H_{10}O_4$: C, 49·3; H, 6·9%). The equivalent weight (78) was found by titration in aqueous alcoholic solution to phenol-red (calc., 73). Rupe, Schobel, and Abegg (*Ber.*, 1912, **45**, 1533) record m. p. 79° for α-methylglutaric acid.

Polymer from Toluquinhydrone.—Toluquinone (Clark, *Amer. Chem. J.*, 1892, **14**, 565) was converted into toluquinol by zinc dust and dilute hydrochloric acid. Toluquinhydrone, obtained by mixing equimolecular quantities of toluquinol and toluquinone, was polymerised by boiling 2N-hydrochloric acid for several hours and also by dissolving it in 2N-sodium hydroxide, warming, and acidifying, the products being apparently identical. The polymer and that from asperulo-

side exhibited identical peaks in the infra-red at 3470s, 1709m, 1639m, 1603m, 1466s, 1374s, 1333w, 1277w, 1215w, 1171w, 1122w, 1120w, 1093m, 1048w, 978w, and 723w cm.$^{-1}$.

Infra-red Spectrum of Aucubin.—We are greatly indebted to Professor P. Karrer, Zürich, for a sample of aucubin, whose infra-red spectrum was measured at an early stage of this investigation when the possibility of asperuloside's being a furan derivative was considered. Bands appeared at 3356s, 1362s, 1348m, 1292w, 1258w, 1238m, 1157m, 1131m, 1109w, 1088s, 1052s, 1026s, 1008s, 965m, 915w, 866w, 850m, 808w, 775w, and 755m cm.$^{-1}$.

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