939. *Diterpenoid Bitter Principles. Part IV.* Investigations on the Constitution of Palmarin.*

By D. H. R. Barton, K. H. Overton, and A. Wylie.

Evidence for the presence of four bitter principles, columbin, chasmanthin, palmarin, and jateorin, in Colombo root has been secured. Alkali-induced isomerisation of chasmanthin affords palmarin, whilst under the same conditions jateorin gives isojateorin. Palmarin and isojateorin are readily obtained pure, and the relation between them has been shown to be one of simple stereoisomerism in the side chain. The functional groups of palmarin (and isojateorin) have been characterised as two lactone groups, one bearing a tertiary α-hydroxyl group, also one β-substituted furan ring and one ethereal ring. The relation of palmarin to columbin has been discussed and they have been proved to possess the same side chain.

In the first two parts of this series [1] the main bitter principle, columbin, of the root of *Jateorhiza palmata* Miers (Colombo root) was shown to have the constitution (I). Various other bitter principles have been described in the literature,[2-7] but the only one of these which was undoubtedly obtained homogeneous is palmarin, $C_{20}H_{22}O_7$. This is a very minor constituent of the root extract, but becomes readily available if the mixed bitter principles, after separation of columbin, are treated with alkali. Clearly the palmarin is derived from a precursor chasmanthin, also $C_{20}H_{22}O_7$, which was isolated by the earlier workers in varying states of purity. The confused nature of the literature [2-7] on chasmanthin is due to the fact that it is admixed with an isomer from which neither we nor, in our opinion, the earlier workers were able to separate it. After controlled treatment with alkali not only can palmarin be obtained pure but also a new bitter principle, for which we propose the name isojateorin. We consider that isojateorin, $C_{20}H_{22}O_7$, is formed by isomerisation of a further isomer jateorin which occurs naturally in admixture with chasmanthin and forms therewith mixed crystals in a most tenacious manner. Since, as will be shown in the sequel, the relation between palmarin and isojateorin is a simple one, conclusions can still be reasonably drawn from the earlier literature [2-7] on " chasmanthin." We shall write this form of designation when the use of a mixture of chasmanthin and jateorin is implied. The results of Wessely and Schonol [3] can be interpreted if their chasmanthin A, contrary to their opinion, is palmarin and their chasmanthin B our isojateorin.

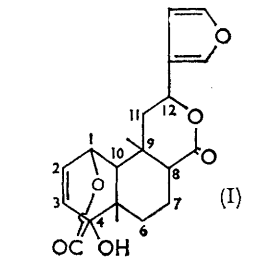
We consider first the chemistry of palmarin and, for ease of exposition, we shall use immediately the formula (II; R = H) which best expresses the results of our experiments.

* Part III, *J.*, 1961, 5061.

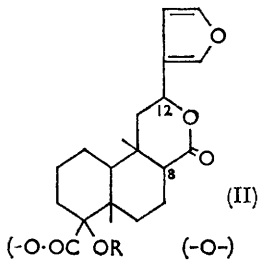
[1] Barton and Elad, *J.*, 1956, 2085, 2090.
[2] Wessely, Schonol, and Isemann, *Monatsh.*, 1936, **68**, 21.
[3] Wessely and Schonol, *Monatsh.*, 1938, **71**, 10.
[4] Feist, Rintelen, and Kuntz, *Annalen*, 1935, **517**, 119.
[5] Feist, Kuntz, and Brachvogel, *Annalen*, 1935, **519**, 124.
[6] Feist and Brachvogel, *Annalen*, 1936, **522**, 185.
[7] Feist, Brachvogel, and Volksen, *Annalen*, 1936, **523**, 289.

Palmarin, like columbin, contains a furan ring readily detected by spectroscopic methods. It has two lactone rings [infrared bands at **1775** (lactone A) and **1710** (lactone B) cm.$^{-1}$] which can be opened reversibly with alkali. Palmarin is also like columbin in that it has a tertiary hydroxyl group which can be methylated with dimethyl sulphate and alkali. This hydroxyl group shows an infrared band at **3490** cm.$^{-1}$ which disappears in the methyl derivatives (II; R = Me). The furan ring was shown to bear one substituent by ozonolysis which afforded trisnorpalmarinic acid (III; R = R′ = H), characterised as its methyl ester (III; R = H, R′ = Me). The attachment of the substituent at the β-position has been established by nuclear magnetic resonance studies upon which we shall report in the next paper of this series.

Reduction of trisnorpalmarinic acid with lithium aluminium hydride gave a hemiacetal (IV) which consumed 2 mol. of periodate to furnish the keto-formate (V), characterised by bands at **1710** (cyclohexanone) and **1730** and **1148** (formate) cm.$^{-1}$. Oxidation with chromic acid gave the δ-lactone (VI; R = CHO) having infrared bands at **1730** (δ-lactone and formate), **1710** (cyclohexanone), and **1148** (formate) cm.$^{-1}$. These experiments establish a partial formulation for the side chain and show that an α-hydroxylactone system, like that in columbin, is also present in palmarin.



(I)



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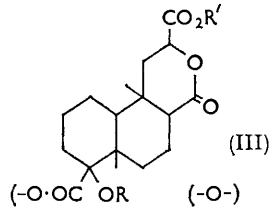

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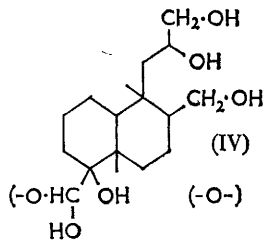
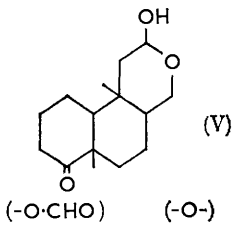


(IV)



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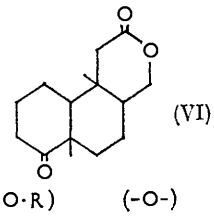
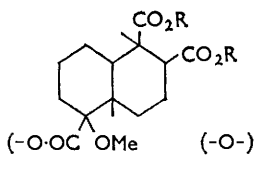


(VI)



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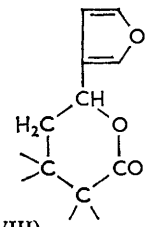

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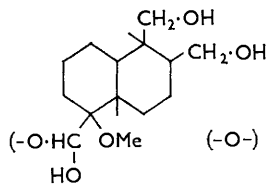


(IX)

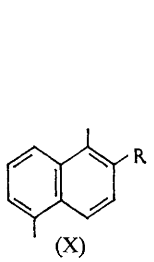
Further information about the side chain of palmarin can be obtained from a consideration of some earlier experiments by Feist and his colleagues.[6,8] Methylpalmarin (designated in the earlier work [6,8] as " methylchasmanthin ") was oxidised by potassium permanganate to " merochasmanthinic acid," a dicarboxylic acid of the constitution $C_{16}H_{20}O_8$. This compound retains the A-lactone of palmarin ($\nu_{max.}$ at **1765** cm.$^{-1}$), but the B-lactone as well as the furan ring have been replaced by two carboxyl groups. On

[8] Feist and Volksen, *Annalen*, 1938, **534**, 41.

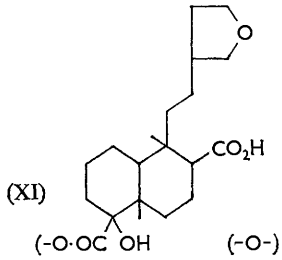
the basis of formula (II) for palmarin " merochasmanthinic acid " can be formulated as (VII; R = H). We have shown that methyltrisnorpalmarinic acid (III; R = Me, R′ = H), isolated as its methyl ester (III; R = R′ = Me), is also formed in this oxidation. If we accept that the B-ring lactone of palmarin is six-membered and that the higher lactone frequency in all derivatives of palmarin represents the A-ring lactone (cf. columbin [1]), then these oxidative degradations prove that the side chain of palmarin is (VIII), identical with that present in columbin (I). We have also reduced " merochasmanthinic acid " with lithium aluminium hydride to the expected hemiacetal (IX).

Palmarin contains an inert oxygen atom which is ethereal in character. This ether must be cyclic; it is indicated in all the relevant formulæ given above.

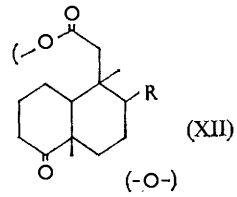
Feist *et al.*[5-7] carried out a number of experiments on the drastic degradation of palmarin (" chasmanthin ") which provide valuable information on the carbon skeleton. Hydrogenation of " chasmanthin " gave a hexahydro-acid (see further below) which on dehydrogenation with selenium furnished 1,2,5-trimethylnaphthalene (X; R = Me) and a so-called lactone, later identified [1] as 1,5-dimethyl-2-naphthoic acid (X; R = CO_2H). Both compounds are obtained by an analogous degradation of columbin.[1] Oxidation of the hexahydro-acid from " chasmanthin " with manganese dioxide and sulphuric acid afforded [5] benzene-1,2,3-tri- and -1,2,3,4-tetracarboxylic acid. The same two acids were obtained in a comparable degradation of columbin (see ref. 1). Distillation of " merochasmanthinic acid " with zinc gave the hydrocarbon (X; R = Me) as well as a little *o*-cresol. *o*-Cresol is readily obtained as a degradation product of columbin.[1] All these results provided strong support for a bicyclic decalin skeleton of the type present in columbin (I) and already used in the palmarin formula (II) given above. By Kuhn–Roth *C*-Me determination and by quantitative infrared spectroscopy as for columbin,[1] the presence of two *C*-methyl groups has been established in palmarin. This is confirmed by nuclear magnetic resonance studies to be published in Part V, which in addition show that both of these methyl groups are attached to quaternary carbon atoms [see (II)].



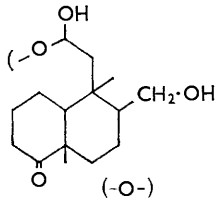
(X)



(XI)



(XII)



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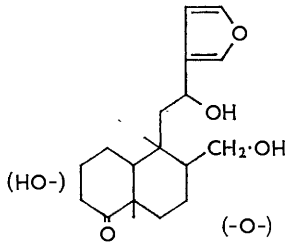
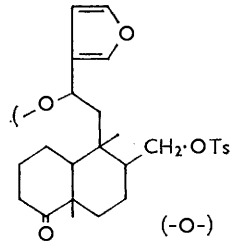


(XIV)



(XV) Ts = *p*-$C_6H_4Me·SO_2$

Hydrogenation of palmarin afforded a mixture of tetrahydropalmarin (as II; furan ring saturated) and, as main product, the known [2] hexahydropalmarinic acid (XI) (see also above). Tetrahydropalmarin showed infrared bands at 3380 (hydroxyl) and 1767 and 1730 (lactones) cm.$^{-1}$. The acid (XI) had a broad infrared band at 1720 cm.$^{-1}$ (carboxyl), indicating a loss of the B-lactone group, but retained the high-frequency

A-lactone band at 1770 cm.$^{-1}$. A comparable hydrogenation of isojateorin furnished tetrahydroisojateorin, which was not identical with tetrahydropalmarin, and a hexahydro-acid identical with hexahydropalmarinic acid (XI). The sole difference between palmarin and isojateorin therefore lies in the stereochemistry at C-12 [see (II)]. This conclusion was confirmed by oxidation of methylisojateorin with potassium permanganate which gave " merochasmanthinic acid " (VII; R = H).

Columbin is readily converted by alkali into isocolumbin. This isomerisation involves simply epimerisation at C-8. The same experimental conditions will convert the chasmanthin–jateorin mixture into palmarin and isojateorin. We consider that the relation between these pairs of bitter principles is, therefore, comparable and involves only epimerisation at C-8 [in (II)].

The lactone (VI; R = CHO) was saponified under very mild conditions, to give a compound whose analyses agreed with those for the expected hydroxy-lactone (VI; R = H) and which gave a monoacetyl derivative. It showed infrared bands indicating the presence of cyclohexanone- and δ-lactone-carbonyl groups. However, it was oxidised smoothly by chromic acid to an acid, $C_{15}H_{18}O_6$, characterised as its methyl ester. The saponification product must, therefore, contain a primary hydroxyl group. From the degradational sequence employed this primary hydroxyl must represent the original B-lactone of palmarin. Therefore, the hydroxyl group of compound (VI; R = H) must have disappeared by lactonisation to give a new lactone. The acid $C_{15}H_{18}O_6$ is represented as (XII; R = CO_2H) without final commitment as to the point of attachment of its lactone ring. This acid was also conveniently obtained by mild saponification of the keto-formate (V) to give a product formulated as (XIII), which on chromic acid oxidation afforded the acid (XII; R = CO_2H).

Reduction of palmarin with lithium aluminium hydride gave the expected hemiacetal. This was not obtained crystalline but on cleavage with periodate and mild hydrolysis with base it gave a crystalline trihydroxy-ketone (XIV). With toluene-*p*-sulphonyl chloride this furnished a monotoluene-*p*-sulphonate which contained no hydroxyl group and gave analyses as for a monoanhydro-compound. By analogy with the compounds (XII; R = CH_2·OH) and (XIII) this can be formulated as (XV).

Further studies on the constitution of palmarin are in hand.

Experimental

M. p.s were taken on the Kofler block. Unless specified to the contrary, $[\alpha]_D$ refer to $CHCl_3$, and ultraviolet absorption spectra to EtOH solutions. Infrared absorption spectra were for Nujol suspensions unless stated otherwise. The analytical data are due to Mr. J. M. L. Cameron (Glasgow) and his colleagues. Alumina for chromatography was acid-washed, neutralised, ignited, and then standardised according to Brockmann and Schodder.[9] The silica gel for chromatography was supplied by B.D.H. Light petroleum refers to the fraction of b. p. 60—80° unless stated otherwise.

Separation of the Bitter Principles.—The following procedure represents the most convenient method of fractionation from many that were investigated. The root extract (total solids obtained by ether-extraction; 200 g.) was heated with acetone (2 l.) to the b. p. and ethanol (1·4 l.) added. The mixed solvent was concentrated (to 3 l.), further ethanol (600 ml.) added, and the process repeated. The solution was cooled to 20°, then left for 2 hr. at this temperature, and the small (amorphous) precipitate removed by filtration (Supercel). Ethanol (1 l.) was added and the solution again concentrated (to 3·5 l.). Further ethanol (500 ml.) was again added and the process repeated (to 3·4 l.). On storage at room temperature overnight crude columbin (30—40 g.) separated. This was filtered off and washed with ethanol (300 ml.). The combined filtrates were concentrated (to 3 l.) and again set aside, to furnish a crude " chasmanthin "–columbin mixture (about 40 g.). This was removed and washed with ethanol (300 ml.), and the combined filtrates were again concentrated (to 2·5 l.). Keeping the solution for 1—2 days at room temperature then gave crude " chasmanthin " (about 40 g.).

[9] Brockmann and Schodder, *Ber.*, 1941, **74**, 73.

The two " chasmanthin "-containing fractions were combined and the columbin contained therein was decarboxylated in the following way. The mixture (15 g., most convenient scale) was heated at 240—250° for 7—10 min. (evolution of carbon dioxide essentially complete), cooled, and dissolved in acetone (200 ml.) *before* solidification. Addition of benzene (200 ml.) and concentration of the solution (to ~125 ml.) furnished " chasmanthin " (4 g.). A typical specimen had m. p. 225—228° (sinters at 220°), $[\alpha]_D$ 0° (*c* 2·05 in pyridine). Repeated crystallisation from acetone–benzene gave palmarin in very small amount.

Palmarin was readily obtained from " chasmanthin " by the following procedure. " Chasmanthin " (20 g.), suspended in refluxing ethanol (280 ml.), was treated with aqueous N-sodium hydroxide (140 ml.) on the steam-bath. After dissolution (3 min.), heating was continued for 2 min. The alkaline solution was cooled, filtered through Supercel, acidified by 2N-hydrochloric acid, and left overnight. The crude palmarin was filtered off and crystallised from acetone–ethyl acetate. This gave good-quality material (10—12 g.), m. p. 253—258°, $[\alpha]_D$ +17° (*c* 1·45 in pyridine) (Found: C, 64·4; H, 5·8; *C*-Me, 7·2. Calc. for $C_{20}H_{22}O_7$: C, 64·2; H, 5·9; 2*C*-Me, 8·0%). The *C*-Me content of isocolumbin was determined at the same time (Found: *C*-Me, 7·5. Calc. for $C_{20}H_{24}O_6$: 2*C*-Me, 8·4%). Palmarin (216 mg.) in ethanol (2 ml.) and aqueous N-sodium hydroxide (8 ml.) was heated on the steam-bath for 5 hr. (uptake 2·3 mol. of alkali). Acidification gave back unchanged palmarin (m. p., mixed m. p., and rotation).

Removal of the solvent from the mother-liquors remaining after the crystallisation of palmarin (see above) gave a residue (soluble in cold ethyl acetate). Crystallisation of this from methanol then afforded *isojateorin* as plates, m. p. 165—167°, $[\alpha]_D$ +30° (*c* 3·99 in pyridine) (Found: C, 63·9; H, 6·3. $C_{20}H_{22}O_7$ requires C, 64·15; H, 5·9%). Isojateorin showed infrared bands at 3460 (OH), 1715 to 1765 (not resolved; two lactones) cm.$^{-1}$, and $\nu_{max.}$ in $CHCl_3$ at 1774 and 1747 (two lactones) cm.$^{-1}$.

Derivatives of Palmarin and Isojateorin.—Palmarin was methylated with dimethyl sulphate and alkali as described earlier.[2] Recrystallised from aqueous acetone, methylpalmarin had m. p. 261—263°, $[\alpha]_D$ +50° (*c* 1·71 in pyridine).

Isojateorin (427 mg.) was methylated as for palmarin (see above), to give *methylisojateorin*. Recrystallised from ethanol as rods (240 mg.), this had m. p. 275—278° (after sintering at 272°), $[\alpha]_D$ +58° (*c* 1·40 in pyridine), $\nu_{max.}$ 1740 and 1773 (two lactones) cm.$^{-1}$, $\nu_{max.}$ (in $CHCl_3$) 1778 and 1755 (two lactones) cm.$^{-1}$ (Found: C, 64·7; H, 6·35. $C_{21}H_{24}O_7$ requires C, 64·9; H, 6·2%).

Methylpalmarin (212 mg.), heated with aqueous alkali as described above for palmarin (uptake 1·97 mol.), was recovered unchanged (m. p., mixed m. p., and rotation) on acidification.

Oxidation of Methylpalmarin with Potassium Permanganate.—Methylpalmarin (2·08 g.) was oxidised with aqueous potassium permanganate as described by Feist and Brachvogel [6] to give " merochasmanthinic acid " (240 mg.), as needles (from acetone–benzene), m. p. (in evacuated capillary) about 228° (decomp.), resolidification, and then 248—251° (decomp.), $[\alpha]_D$ +46° (*c* 1·06 in pyridine) (Found: C, 56·95; H, 5·85. Calc. for $C_{16}H_{20}O_8$: C, 56·45; H, 5·95%). A better yield of " merochasmanthinic acid " was obtained by the following procedure. Methylpalmarin (1·07 g.) in acetone (33 ml.) was treated with aqueous N-sodium hydroxide (11 ml.) on the steam-bath for 7 min. The acetone was removed *in vacuo* and manganese sulphate (50 mg.) added. The solution, cooled to room temperature, was treated dropwise during 20 min. with stirring with 5% aqueous potassium permanganate (210 ml.), and the stirring continued for 2 hr. The excess of oxidant was destroyed (at 0°) with sulphur dioxide and, after saturation with sodium chloride, the acid was extracted with ether. Crystallisation of the product from acetone–benzene gave the desired acid (487 mg.).

Methylation of the acidic material remaining in the mother-liquors after removal of the " merochasmanthinic acid " gave, after crystallisation from acetone–light petroleum *methyl methyltrisnorpalmarinate* as needles, m. p. 260—263°, $[\alpha]_D$ +60° (*c* 1·34 in $CHCl_3$) (Found: C, 60·5; H, 6·3. $C_{19}H_{24}O_3$ requires C, 60·0; H, 6·35%). Chromatography of the residual methylation product over alumina (grade III), and elution with benzene and benzene–ether, afforded " methyl merochasmanthinate " as plates (from acetone–light petroleum), m. p. (sublimes) 240—245°, $[\alpha]_D$ +64° (*c* 1·21 in pyridine), +80° (*c* 3·38 in $CHCl_3$) (Found: C, 58·75; H, 5·9; OMe, 22·85. Calc. for $C_{18}H_{24}O_8$: C, 58·65; H, 6·55; OMe, 25·3%). The same compound was obtained by treating " merochasmanthinic acid " with diazomethane.

Oxidation of methylisojateorin (130 mg.) with potassium permanganate by the method

(see above) due to Feist and Brachvogel [6] gave " merochasmanthinic acid " (11 mg.), identified by m. p., mixed m. p., and infrared spectrum. Methylation of the residual acid in the mother-liquors furnished " methyl merochasmanthinate " (16 mg.), identified by m. p., mixed m. p., and infrared spectrum.

Hydrogenation of Palmarin.—Palmarin (322 mg.) in acetic acid (115 ml.) was hydrogenated over 10% palladised charcoal (60 mg.) until uptake was complete. The product, in dichloromethane, was separated with sodium carbonate solution into acidic and neutral fractions. Crystallisation of the neutral fraction from acetone–light petroleum afforded *tetrahydropalmarin* (40 mg.), m. p. 322—325°, $[\alpha]_D$ +16° (*c* 0·95 in pyridine) (Found: C, 63·25; H, 6·9. $C_{20}H_{26}O_7$ requires C, 63·5; H, 6·95%). The acidic fraction, crystallised from aqueous ethanol, furnished hexahydropalmarinic acid (135 mg.), m. p. 214—216°, $[\alpha]_D$ +41° (*c* 2·16 in pyridine) (Found: C, 63·05; H, 7·5. Calc. for $C_{20}H_{28}O_7$: C, 63·15; H, 7·4%). Hexahydropalmarinic acid (87 mg.) was shaken with 55% aqueous hydriodic acid (3 ml.) at room temperature for 14 hr. The starting material (53 mg.) was recovered unchanged (m. p. and mixed m. p.). The acid resisted hydrogenation over platinum oxide in acetic acid containing 2% of perchloric acid (as 70% aqueous acid).

Hydrogenation of Isojateorin.—Isojateorin (590 mg.) was hydrogenated as for palmarin (see above), to give acidic and neutral fractions. Crystallisation of the latter from acetone gave *tetrahydroisojateorin* (50 mg.), m. p. 318—320°, $[\alpha]_D$ +39° (*c* 0·95 in pyridine) (Found: C, 63·45; H, 7·15. $C_{20}H_{26}O_7$ requires C, 63·5; H, 6·95%). The m. p. was clearly depressed on admixture with tetrahydropalmarin, and the infrared spectra of the two compounds were different. The acidic fraction, crystallised from aqueous ethanol, gave an acid (265 mg.) which was identical (m. p., mixed m. p., rotation, and infrared spectrum) with hexahydropalmarinic acid (see above).

Ozonolysis of Palmarin.—Palmarin (2·336 g.) in ethyl acetate (850 ml.) was ozonised at −70° until ε was about 500 at 215 mμ (7 hr.; appearance of blue colour due to excess of ozone). The solvent was removed *in vacuo* at <40° and the residue taken up in water, filtered from a small amount of insoluble residue, and kept at room temperature for 16 hr. The desired *trisnorpalmarinic* acid separated as rods and, recrystallised from ethanol (1·725 g.), had m. p. 270—272° (crystals became opaque at about 180°) (Found: C, 57·55; H, 5·9. $C_{17}H_{20}O_8$ requires C, 57·95; H, 5·7%). Treatment of the acid in acetone solution with ethereal diazomethane gave the *methyl ester*. Recrystallised from ethanol this had m. p. 263—265° (Found: C, 58·5; H, 6·65. $C_{18}H_{22}O_8,C_2H_6O$ requires C, 58·25; H, 6·85%).

Reduction of Trisnorpalmarinic Acid with Lithium Aluminium Hydride.—The trisnor-acid (1·22 g.) in dry tetrahydrofuran (100 ml.) was added dropwise with stirring to a refluxing suspension of lithium aluminium hydride (3·5 g.) in the same solvent (50 ml.) during 4·5 hr. and the refluxing continued for 1 hr. more. After cooling to 0° the excess of reductant was destroyed with ethyl acetate, ammonium sulphate solution (saturated aqueous; 10 ml.) was added, and the precipitated aluminium compounds were removed by filtration and washed with hot water (4 × 15 ml.). The combined filtrate was freed from organic solvents *in vacuo* and the resultant aqueous solution was saturated with ammonium sulphate and continuously extracted with ether (4 days), to furnish the *hemiacetal* (IV), crystallising as square plates (from methanol–benzene) (722 mg.), m. p. 213—215° (Found: C, 59·2; H, 8·6. $C_{17}H_{28}O_7$ requires C, 59·3; H, 8·2%). This hemiacetal (515 mg.) in water (15 ml.) was treated with sodium metaperiodate (800 mg.) in water (10 ml.) at 20° for 15 min. (rapid uptake of 1·96 mol. of periodate in a preliminary experiment). The *keto-formate* (V) separated as needles. Recrystallised from acetone–hexane this formed hexagonal plates (393 mg.), m. p. 200—202°, $[\alpha]_D$ −22° (*c* 1·30 in acetone) (Found: C, 62·1; H, 6·95. $C_{16}H_{22}O_6$ requires C, 61·9; H, 7·15%).

The keto-formate (490 mg.) in " AnalaR " acetic acid (5 ml.) was treated with a 0·27N-solution (15 ml.) of chromium trioxide in aqueous acetic acid (1 : 19) for 16 hr. Methanol (5 ml.) was added and the combined solvents were removed *in vacuo*. Crystallisation of the product from aqueous ethanol afforded the *lactone* (VI; R = CHO) (423 mg.) as fine needles, m. p. 233—236° (Found: C, 62·5; H, 6·6. $C_{16}H_{20}O_6$ requires C, 62·3; H, 6·55%). This lactone (VI; R = CHO) (129 mg.) in methanol (5 ml.) was treated with potassium carbonate (700 mg.) in water (5 ml.) at 20° for 16 hr. The methanol was removed *in vacuo* at room temperature, and the residual aqueous solution saturated with sodium dihydrogen phosphate and extracted with chloroform. Crystallisation of the product from acetone–benzene gave the *hydroxy-lactone* (XII; R = CH_2·OH) (111 mg.) as oblong prisms, m. p. 255—258°, $[\alpha]_D$ +43°

(*c* 1·26 in acetone) (Found: C, 64·35; H, 7·2. $C_{15}H_{20}O_5$ requires C, 64·25; H, 7·2%). Treatment with pyridine–acetic anhydride overnight at room temperature furnished the corresponding *acetate* (XII; R = CH_2·OAc). Recrystallised from aqueous ethanol this had m. p. 196—198°, resolidifies, 213—215° (Found: C, 63·35; H, 6·8. $C_{17}H_{22}O_6$ requires C, 63·35; H, 6·9%).

The lactone (XII; R = CH_2·OH) (123 mg.) in acetic acid (2 ml.) was treated with a 0·078N-solution (23·1 ml.) of chromium trioxide in aqueous acetic acid (1 : 19) overnight at room temperature. The product was separated into neutral (7 mg.) and acidic (107 mg.) fractions. Crystallisation of the latter from ethanol gave the *acid* (XII; R = CO_2H), m. p. 268—270°, pK_a 4·42 (Found: C, 61·2; H, 6·2. $C_{15}H_{18}O_6$ requires C, 61·2; H, 6·15%). Methylation with ethereal diazomethane furnished immediately the *methyl ester* (XII; R = CO_2Me), m. p. 198—200° (from ethyl acetate–light petroleum) (Found: C, 62·1; H, 6·6. $C_{16}H_{20}O_6$ requires C, 62·3; H, 6·55%).

The same acid (XII; R = CO_2H) was obtained more directly by the following route. The keto-formate (V) (150 mg.) and potassium carbonate (2·0 g.) in 1 : 1 v/v aqueous methanol (10 ml.) were kept at 20° for 16 hr. The solution was saturated with sodium dihydrogen phosphate and extracted with chloroform, to give a product (XIII) (138 mg.) which formed needles (from ethyl acetate–light petroleum), m. p. 174—178°. Without further purification this product (84 mg.) in " AnalaR " acetic acid (5 ml.) was treated with a 0·124N-solution (9·6 ml.) of chromium trioxide in aqueous acetic acid (1 : 19) for 3 hr. Separation of the product into neutral and acidic fractions gave in the latter the acid (XII; R = CO_2H) (47 mg.) described above (m. p., mixed m. p., and infrared spectrum). The identity was confirmed by conversion into the methyl ester (m. p., mixed m. p., and infrared spectrum). The neutral fraction, crystallised from acetone–light petroleum, gave a *compound* (24 mg.) as rods, m. p. 218—220° (Found: C, 64·3; H, 6·5. $C_{15}H_{18}O_5$ requires C, 64·7; H, 6·5%).

Reduction of Palmarin with Lithium Aluminium Hydride.—Palmarin (510 mg.), suspended in dry tetrahydrofuran (60 ml.), was added gradually to lithium aluminium hydride (1·0 g.) in refluxing tetrahydrofuran (15 ml.) and refluxing continued for 1 hr. The product, worked up essentially as in the reduction described above and isolated by continuous ether-extraction, was a gum (489 g.) showing no carbonyl absorption in the infrared spectrum. It was kept for 4 hr. in a mixture of tetrahydrofuran (5 ml.) and aqueous 0·25M-periodic acid (10 ml.) adjusted to pH 7 with saturated sodium hydrogen carbonate solution. The product (322 mg.), isolated in the usual way, was kept in methanolic 2% potassium hydroxide (5 ml.) for 5 min. On dilution with water and acidification the *trihydroxy-ketone* (XIV) separated as rosettes of needles (240 mg.). Recrystallised from ethanol–benzene this had m. p. 196—198°, $[\alpha]_D$ −40° (*c* 1·26 in pyridine), $\nu_{max.}$ 3380 (OH) and 1710 (cyclohexanone) cm.$^{-1}$ (Found: C, 65·25; H, 7·2. $C_{19}H_{26}O_6$ requires C, 65·1; H, 7·5%). In a second experiment the acidified (dilute sulphuric acid) solution was steam-distilled and the volatile acid produced converted into its sodium salt and identified by its infrared spectrum as sodium formate.

This trihydroxy-ketone (100 mg.) in dry pyridine (3 ml.) was kept overnight with toluene-*p*-sulphonyl chloride (recrystallised; 600 mg.). Working up in the usual way, chromatography over alumina (grade III), and elution with benzene furnished the *toluene*-p-*sulphonate* (XV), m. p. 163—165° (from benzene–light petroleum), $\lambda_{max.}$ 223 mμ (ε 14,800), $\nu_{max.}$ 1705 (cyclohexanone) and 1595 (toluene-*p*-sulphonate) cm.$^{-1}$ (Found: C, 64·0; H, 6·0; S, 6·8. $C_{26}H_{30}O_7S$ requires C, 64·2; H, 6·2; S, 6·6%).

Reduction of " Merochasmanthinic Acid " with Lithium Aluminium Hydride.—" Merochasmanthinic acid " (290 mg.) was continuously extracted from a Soxhlet thimble into ether (300 ml.) containing lithium aluminium hydride (2·4 g.) during 20 hr. Working up in the usual way gave a product (190 mg.) which on treatment with ether furnished crystalline material (28 mg.). Recrystallised from acetone–light petroleum this had m. p. 178—180° (Found: C, 61·15; H, 8·0. $C_{16}H_{26}O_6$ requires C, 61·15; H, 8·35%). It showed no infrared carbonyl band and is therefore formulated as the *hemiacetal* (IX).

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