61. *Constituents of* " Cortex Piscidiæ Erythrinæ." *Part I.* *The Structure of Piscidic Acid.*

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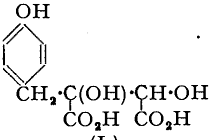
Piscidic acid has been shown to be a trihydroxy-dibasic acid, forming acid and neutral esters and an *O*-dimethyl ether. The hydroxyl groups have been proved to be respectively phenolic and secondary and tertiary alcoholic, of which the latter pair are attached to adjacent carbon atoms. Oxidation of *O*-dimethylpiscidic acid and application of the Weerman reaction to the corresponding diamide gave anisaldehyde, whilst degradation of methyl *O*-methylpiscidate by means of lead tetra-acetate yielded *p*-methoxyphenylpyruvic acid. Piscidic acid is shown to have formula (I).

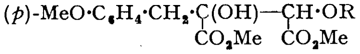
The chemical constituents of this drug, which is the root bark of the tree *Piscidia erythrina* (Linn.) (" Jamaica Dogwood ") and was used as a hypnotic and narcotic, have been examined by Hart (*J. Amer. Chem. Soc.*, 1883, **5**, 39), Swaters (*Nederl. Tijdschr. Pharm.*, 1896, **8**, 39), Freer and Clover (*Amer. Chem. J.*, 1901, **25**, 390), Dankwortt and Schütte (*Arch. Pharm.*, 1934, **272**, 701), and Hauschild (*ibid.*, 1936, **274**, 388). Of these the best defined product is the dibasic acid, piscidic acid, $C_{11}H_{12}O_7$, first isolated and examined by Freer and Clover, who prepared a monoethyl ester and its amorphous acetate and also a dianilide, concluding that the substance did not contain an aromatic nucleus and was a dihydroxy-dicarboxylic acid of the aliphatic series in which the carboxyl groups were probably attached to adjacent carbon atoms (positive fluorescein reaction).

Piscidic acid has been isolated by a modification of Freer and Clover's procedure, and their conclusions regarding its empirical formula and basicity have been confirmed. The compound, which does not appear to lose carbon dioxide readily on being heated, has been found to be optically active, does not contain a methoxyl group, is stable to boiling 30% aqueous sodium hydroxide, and behaves as a saturated substance towards hydrogen and a palladium catalyst. Though the acid slowly reduces hot Fehling's solution, all attempts to detect the presence of a carbonyl group gave negative results. By the usual methods it gives *mono-* and *di-methyl* esters of which only the latter gives a ferric reaction, indicating the presence of a phenolic hydroxyl group in agreement with the solubility of the dimethyl ester in dilute aqueous sodium hydroxide; the negative ferric reaction given by the acid and the acid esters appears to be due to the effect of the free carboxyl groups. In the absence of a carbonyl group, conclusive proof that there is a phenolic residue in piscidic acid is afforded by the fact that the acid and its esters react with benzenediazonium chloride in alkaline solution, forming red-brown azo-dyes. Further, the conclusion that piscidic acid contains a benzenoid system is in agreement with the empirical formula and the absence of an easily hydrogenated ethylenic system.

On methylation by the methyl iodide–potassium carbonate method, methyl piscidate invariably gave a *dimethyl ether* of the ester which on hydrolysis yielded *O-dimethylpiscidic acid*. Oxidation of the latter compound with potassium permanganate gave anisic acid, thus showing that the dimethyl ether is a derivative of anisole substituted in the *p*-position by the group $C_3H_4O(OMe)(CO_2H)_2$ (A), and in the circumstances it appeared that the remaining oxygen atom unaccounted for in the residue (A) was present in an ether system or as an alcoholic hydroxyl group. The presence of the latter group in (A) was quantitatively demonstrated by the application of the Zerewitinoff estimation to methyl *O*-dimethylpiscidate, and, since methyl piscidate gives a tri-acetate but is not apparently converted readily into a trimethyl ether by the methyl iodide–silver oxide method (an amorphous product was obtained, the methoxyl content of which agreed with a dimethyl ether of methyl piscidate), it seemed likely that this hydroxyl group is tertiary, a conclusion in agreement with the positive mercuric sulphate test for a tertiary alcohol given by methyl *O*-dimethylpiscidate. Further, the other alcoholic hydroxyl group present in the side chain is easily methylated, but, as attempts to form a trityl ether of methyl piscidate were unsuccessful, it appeared that this group was secondary, and, moreover, since methyl piscidate readily condenses with acetone in the presence of a dehydrating agent to give an iso*propylidene* derivative, it is reasonably certain that the hydroxyl groups are attached to adjacent carbon atoms. Similarly, the positive fluorescein reaction given by piscidic acid (Free and Clover, *loc. cit.*), which we have confirmed, indicates that the carboxyl groups are also attached to adjacent carbon atoms, and, as it is clear that these groups are contained in the side chain, it seemed likely that piscidic acid was a substituted succinic acid of the type (I) for which there are a number of possible structures.

In the course of numerous degradation experiments carried out to determine the exact structure of the side chain of piscidic acid it was found that the action of hydriodic acid gave only small amounts of an individual substance whilst application of Fenton's reaction, the Oppenauer reaction, and oxidation with lead tetra-acetate or periodic acid to methyl piscidate gave only traces of tractable degradation products. On the other hand the Weerman reaction applied to the *diamide* of *O*-dimethylpiscidic acid furnished anisaldehyde, but all attempts to isolate an intermediate oxidation product were unsuccessful. Similarly, anisaldehyde was also formed when methyl *O*-dimethylpiscidate was oxidised with chromic oxide. Since it seemed reasonably certain that piscidic acid was a 1 : 2-glycol which normally would be expected to be degraded with lead tetra-acetate or periodic acid, the failure of methyl piscidate to yield definite products with these reagents appeared to be due to the interference of the free phenolic hydroxyl group. After numerous attempts by other methods it was found that methyl piscidate could be mono-methylated or -benzylated by the action of methyl iodide or benzyl bromide, respectively, on its sodio-derivative, thus giving *monomethyl* and *monobenzyl ethers* of methyl piscidate which are insoluble in aqueous sodium hydroxide. Oxidation of the monomethyl ether with lead tetra-acetate in acetic acid gave rise to a crystalline compound, the nature of which has not yet been fully investigated, but which on hydrolysis with boiling alcoholic potassium hydroxide gave *p*-methoxyphenylpyruvic acid (III, R = Me) with a small amount of anisaldehyde. Similarly, oxidation of the monobenzyl ether yielded a crystalline product which on being hydrolysed gave p-*benzyloxyphenylpyruvic acid* (III, R = $Ph{\cdot}CH_2$), identical with an authentic specimen. A more detailed examination of the optically inactive primary oxidation products formed by lead tetra-acetate will be made when material is available.

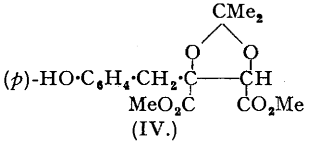

(I.)


$(p)\text{-}MeO{\cdot}C_6H_4{\cdot}CH_2{\cdot}\underset{CO_2Me}{C}(OH)\text{—}\underset{CO_2Me}{C}H{\cdot}OR$

(II.)

$RO{\cdot}C_6H_4{\cdot}CH_2{\cdot}CO{\cdot}CO_2H$

(III.)


(IV.)

The production of (III, R = Me) from (II, R = H) by lead tetra-acetate clearly indicates the presence of a *p*-hydroxybenzyl residue in piscidic acid, and hence, in agreement with the view that the side chain is a substituted succinic acid residue in which two hydroxyl groups have been shown to be attached to adjacent carbon atoms, it seems reasonably certain that methyl *O*-methylpiscidate has formula (II, R = H). Consequently piscidic acid is *p*-hydroxybenzyltartaric acid (I), forming the dimethyl ether (II, R = Me) and the *iso*propylidene derivative (IV) of the methyl ester.

EXPERIMENTAL.

Piscidic Acid.—Pulverised root bark of *Piscidia erythrina* (1350 g.) was extracted with water (13·5 l. (agitate) for 4 days and the filtered extract concentrated to one-fourth of its volume, treated with charcoal, and filtered. Sufficient saturated lead acetate solution was added to this liquor to precipitate a small amount of colouring matter as a dark brown solid, leaving a pale straw-coloured liquid which on filtration and subsequent treatment with an excess of the reagent gave a precipitate of the almost colourless lead salt of piscidic acid. The solid was washed by decantation, collected, suspended in alcohol, and decomposed with excess of hydrogen sulphide and, after removal of the lead sulphide by filtration, the alcoholic liquor was evaporated under reduced pressure, leaving crude piscidic acid as a straw-coloured crystalline solid contaminated with resinous material. A solution of this product in a slight excess of boiling ethyl acetate was treated with a little warm chloroform, and, after having been decanted from the resinous material which first separated, the hot liquid was treated with an equal volume of boiling chloroform; the optimum proportions of ethyl acetate and chloroform were found by trial for each batch of crude acid. On cooling, the solution deposited piscidic acid in elongated colourless prisms which on recrystallisation had m. p. 183—184° and, after having been dried in a high vacuum at 110—115°, m. p. 186—187°, $[\alpha]_D^{20°}$ +41·03° (*c*, 2·65 in water) (Found: C, 51·0; H, 5·0; *M*, 258. Calc. for $C_{11}H_{12}O_7$: C, 51·6; H, 4·7%; *M*, 256) (Freer and Clover, *loc. cit.*, give m. p. 182—185°).

On being boiled with alcohol (50 c.c.) containing 10 drops of concentrated hydrochloric acid and subsequent removal of the solvent, piscidic acid (2 g.) gave the monoethyl ester (1·3 g.) which separated from warm water and then ethyl acetate–chloroform in slender colourless needles, m. p. 211—212° (Freer and Clover record m. p. 207—208°) (Found: C, 54·9; H, 5·7. Calc. for $C_{13}H_{16}O_7$: C, 54·9; H, 5·6%). This derivative, which is soluble in alcohol, acetone, or ethyl acetate, and insoluble in chloroform, dissolves in aqueous sodium hydrogen carbonate with evolution of carbon dioxide. Prepared by the same procedure, the *monomethyl* ester formed slender colourless needles, m. p. 179°, from ethyl acetate–chloroform, having similar solubilities (Found in material dried in a high vacuum at 110°: C, 52·6; H, 5·5; *M*, 254. $C_{12}H_{14}O_7$ requires C, 53·1; H, 5·5%; *M*, 270). Interaction of this ester (2·5 g.) with ethereal diazomethane (from 3 c.c. of nitrosomethylurethane) for 5 minutes gave the *dimethyl* ester (2·3 g.), forming colourless prisms, m. p. 127°, from benzene and having a bottle-green ferric reaction in aqueous solution (Found: C, 55·1; H, 5·7; *M*, 294. $C_{13}H_{16}O_7$ requires C, 54·9; H, 5·6%; *M*, 284). The same ester, m. p. and mixed m. p. 127°, was obtained by action of excess of diazomethane on piscidic acid and by esterification of the acid (3 g.) in boiling absolute methyl alcohol (100 c.c.) containing 10 g. of sulphuric acid during 12 hours. Acetylation of this ester (1 g.) with pyridine (2·5 ml.) and acetic anhydride (5 ml.) at room temperature during 36 hours gave a glass which did not crystallise but, on being refluxed with acetic anhydride (6 ml.) and sodium acetate (1 g.) for 20 minutes, yielded the *triacetate* (0·8 g.), forming a mat of slender needles, m. p. 84°, from light petroleum (b. p. 60—80°) and having a negative ferric reaction [Found: C, 55·9; H, 5·6; Ac, 32·2. $C_{13}H_{13}O_4(OAc)_3$ requires C, 55·6; H, 5·4; Ac, 31·4%]. The same product was obtained directly from the ester by means of boiling acetic anhydride and sodium acetate.

A solution of methyl piscidate (1 g.) in 2% aqueous sodium hydroxide (7 ml.) was vigorously agitated with ether (15 ml.) containing (0·5 ml.) of acetic anhydride for 15 minutes, the ether was separated, and the residual liquor extracted several times with fresh solvent. Evaporation of the combined washed and dried ethereal extracts left the *monoacetate* of methyl piscidate which formed elongated rectangular prisms, m. p. 117—118°, from water, having a negative ferric reaction [Found: C, 55·4; H, 5·7; OMe, 18·8; Ac, 13·5. $C_9H_7O_5(OMe)_2(OAc)$ requires C, 55·2; H, 5·2; OMe, 19·0; Ac, 13·2%].

O-*Dimethypiscidic Acid.*—Treatment of methyl piscidate with excess of diazomethane during 5 hours gave only unchanged material, but methylation of the ester (3 g.) in boiling acetone (90 ml.) with methyl iodide (5 ml.) and potassium carbonate (6 g.) for 10 hours, with the addition of more iodide (3 ml.) and carbonate (3 g.) after 5 hours, furnished the *dimethyl ether* (II, R = Me) (1·8 g.) of the ester which separated from methyl alcohol in elongated prisms, m. p. 115°, having a negative ferric reaction [Found: C, 57·8; H, 6·5; OMe, 39·5. $C_{11}H_8O_3(OMe)_4$ requires C, 57·7; H, 6·4; OMe, 39·7%]. Hydrolysis of this dimethyl ether (1 g.) with potassium hydroxide (1·1 g.) in alcohol (10 ml.) and water (10 ml.) at room temperature (agitate) for 4 hours yielded O-*dimethylpiscidic acid* which formed colourless rectangular prisms (0·7 g.), m. p. 177°, from ethyl acetate or light petroleum (b. p. 60—80°), having a negative ferric reaction [Found: C, 55·1; H, 5·7; OMe, 21·6. $C_{11}H_{10}O_7(OMe)_2$ requires C, 54·9; H, 5·6; OMe, 21·8%]. Acidification of the potassium carbonate residues from the foregoing methylation gave small amounts of this acid.

When a solution of methyl O-dimethylpiscidate (2 g.) in methyl alcohol (60 ml.) was saturated with ammonia and kept at room temperature for 4 days and the excess of ammonia and alcohol then removed under reduced pressure, an almost theoretical yield of the *diamide* was obtained. This separated from ethyl acetate or chloroform in squat prisms, m. p. 156° [Found: C, 55·3; H, 6·5; N, 9·6; OMe, 22·1. $C_{11}H_{12}O_3N_2(OMe)_2$ requires C, 55·3; H, 6·4; N, 9·9; OMe, 22·0%].

Methyl O-*Methylpiscidate* (II, R = H).—A solution of methyl piscidate (1 g.) in alcohol (15 ml.), containing sodium ethoxide (from 0·4 g. of sodium) and methyl iodide (1·5 ml.), was refluxed for 2·5 hours and the cooled solution diluted with excess of ether, washed twice with 2% aqueous sodium hydroxide (8 ml.) and then with water, and dried. Removal of the solvent left the *monomethyl ether* which formed elongated rectangular prisms (0·4 g.), m. p. 110—111°, having a negative ferric reaction [Found: C, 56·6; H, 6·0; OMe, 30·9. $C_{11}H_9O_4(OMe)_3$ requires C, 56·4; H, 6·1; OMe, 31·2%]. A lower yield of the same product was obtained when methyl alcohol was used as the solvent, thus showing that there had been no interchange of radical in either of the ester groups by the use of ethyl alcohol as a solvent.

When the methyl iodide was replaced by benzyl bromide (6 ml.) in the foregoing alkylation a good yield of the corresponding *monobenzyl ether* was obtained which, on repeated crystallisation from dilute alcohol, formed slender rods, m. p. 100° (Found: C, 64·3; H, 6·2. $C_{20}H_{22}O_7$ requires C, 64·2; H, 5·9%).

iso*Propylidene Derivative of Methyl Piscidate* (IV).—A mixture of methyl piscidate (1 g.), acetone (25 ml.), and concentrated sulphuric acid (1 ml.) was kept for 3 hours, the resulting red mixture was neutralised with dry sodium hydrogen carbonate, the salts were removed by filtration (wash with acetone), and the solvent was evaporated under reduced pressure. A solution of the amber-coloured residual oil in ether was washed, dried, and evaporated, leaving the iso*propylidene* derivative (0·65 g.) which formed prisms, m. p. 140°, from dilute alcohol or carbon tetrachloride, readily soluble in chloroform, benzene, or ethyl acetate [Found: C, 58·9; H, 6·0; OMe, 18·8. $C_{14}H_{14}O_5(OMe)_2$ requires C, 59·3; H, 6·2; OMe, 19·1%]. Hydrolysis of this compound with warm dilute hydrochloric acid gave acetone which was characterised by the formation of its 2 : 4-dinitrophenylhydrazone, m. p. 124°, identified by comparison with an authentic specimen.

Oxidation of Piscidic Acid Derivatives.—(*a*) To a solution of *O*-dimethylpiscidic acid, obtained by the hydrolysis of the methyl ester (1 g.) with 1% aqueous sodium hydroxide (10 ml.), 3% aqueous potassium permanganate was gradually added during 4 hours until the permanganate colour persisted. Next day the solution was cleared with sulphur dioxide, the liquor was saturated with ammonium sulphate and repeatedly extracted with ether, and the dried ethereal extracts were evaporated, leaving an acid which on crystallisation from water and then benzene gave slender rectangular prisms, m. p. 183—184°, identical in every way with an authentic specimen of anisic acid [Found: C, 63·1; H, 5·3; OMe, 20·3; *M* (by titration), 150·9. Calc. for $C_7H_5O_2(OMe)$: C, 63·2; H, 5·3; OMe, 20·4%; *M*, 152]. Prepared by means of diazomethane, the methyl ester had m. p. 48°, undepressed on admixture with an authentic specimen [Found: C, 65·1; H, 6·1; OMe, 37·1. Calc. for $C_7H_4O(OMe)_2$: C, 65·1; H, 6·0; OMe, 37·4%].

(*b*) Suspended in water (40 ml.) at 0°, the diamide of *O*-dimethylpiscidic acid (1 g.), was treated with aqueous sodium hypochlorite (11 ml. of a solution containing 5·5% of available chlorine) and the mixture maintained at 0° for 2 days. After the removal of unchanged amide (0·35 g.) by filtration, the solution was acidified and extracted several times with ether. Treatment of an alcoholic solution of the oil left on evaporation of the ether with 2 : 4-dinitrophenylhydrazine hydrochloride solution gave the 2 : 4-dinitrophenylhydrazone of anisaldehyde, forming vermilion plates, m. p. 250° (decomp.), identical with an authentic specimen [Found: C, 53·5; H, 3·8; N, 17·8; OMe, 9·8. Calc. for $C_{14}H_{12}O_5N_4$; C, 53·2; H, 3·8; N, 17·7; OMe, 9·8%]. Oxidation of the crude reaction product with aqueous potassium permanganate gave anisic acid, m. p. and mixed m. p. 183—184°.

(*c*) Oxidation of *O*-dimethylpiscidic acid (1 g.) in water (15 ml.) with chromic oxide (0·5 g.) in water (5 ml.) during 2¼ hours with subsequent addition of methyl alcohol (5 ml.) to destroy unchanged chromic acid gave, on isolation of the product with ether, anisaldehyde which was characterised by the formation of its 2 : 4-dinitrophenylhydrazone, m. p. 250° (decomp.).

(*d*) A solution of methyl *O*-methylpiscidate (II, R = H) (1 g.) and lead tetra-acetate (1·55 g.) in acetic acid (25 ml.) was kept for 24 hours, diluted with water (75 ml.), and repeatedly extracted with ether. The combined ethereal extracts were washed with saturated aqueous sodium hydrogen carbonate to remove acetic acid, dried, and evaporated, leaving a colourless crystalline residue (0·8 g.). Recrystallised from benzene or benzene–light petroleum (b. p. 60—80°), this compound formed angular prisms (0·55 g.), m. p. 129°, which had an intense green ferric reaction in alcohol and was optically inactive [Found: C, 60·6; H, 4·6; OMe, 29·4%]. A solution of the compound (1·5 g.) in alcohol (33 ml.) containing potassium hydroxide (1·8 g.) was refluxed for 2¼ hours, acidified with dilute hydrochloric acid, and extracted with ether. After the removal of acidic material from the combined ethereal extracts, the latter were dried and evaporated, leaving a small quantity of anisaldehyde, identified by conversion into the 2 : 4-dinitrophenylhydrazone. Acidification of the sodium hydrogen carbonate washings gave an acid which separated from acetic acid in elongated prisms (0·35 g.), m. p. 179° (decomp.), having an intense green ferric reaction, and was identified as *p*-methoxyphenylpyruvic acid by comparison with an authentic specimen [Found: C, 62·0; H, 5·5; OMe, 16·0. Calc. for $C_9H_7O_3(OMe)$: C, 61·9; H, 5·2; OMe, 16·0%]. This compound formed an oxime which separated from benzene in rectangular prisms, m. p. 157° (decomp.), identical with the oxime of *p*-methoxyphenylpyruvic acid [Found: C, 57·8; H, 5·4; N, 6·8; OMe, 15·1. Calc. for $C_9H_8O_3N(OMe)$: C, 57·4; H, 5·2; N, 6·7; OMe, 14·8%]. The methyl ester, colourless prisms from aqueous methyl alcohol, had m. p. 95°, undepressed on admixture with an authentic specimen.

(*e*) (With J. B. D. McKenzie.) Methyl *O*-benzylpiscidate (1 g.) was oxidised with lead tetra-acetate and the product isolated by the procedure employed in (*d*). Crystallised from benzene, the latter substance was obtained in long slender needles, m p. 157°, soluble in alcohol or ethyl acetate and insoluble in aqueous sodium hydrogen carbonate and having a dark green ferric reaction in alcohol (Found: C, 67·9; H, 5·4%; *M*, 303). Hydrolysis of this substance, which was optically inactive, in boiling 5% aqueous-alcoholic sodium hydroxide for 2 hours yielded p-*benzyloxyphenylpyruvic acid* which, on purification by means of aqueous sodium hydrogen carbonate and then by crystallisation from a little acetic acid or from 50% acetic acid, formed clusters of prisms, m. p. 192°, after sintering at 180—182°, identical with a synthetical specimen prepared by the following method. Hydrolysis of 4-(4′-acetoxybenzylidene)-2-methyl-5-oxazolone (Dakin, *J. Biol. Chem.*, 1929, **82**, 439) (1 g.) with 1·5N-hydrochloric acid (20 ml.) at 100° for 4 hours gave *p*-hydroxyphenylpyruvic acid (0·6 g.), m. p. 220°, after purification (cf. Neubauer and Fromberg, *Z. physiol. Chem.*, 1910, **70**, 326), which on esterification with methyl alcohol and hydrogen chloride gave the methyl ester as a low melting solid, b. p. 125°/0·03 mm. The ester was characterised by the formation of the 2 : 4-*dinitrophenylhydrazone* which separated from ethyl acetate in pale yellow rhombs, m. p. 201° (Found: C, 51·2; H, 3·5; N, 15·1. $C_{16}H_{14}O_7N_4$ requires C, 51·3; H, 3·7; N, 15·0%). Prepared by the benzyl bromide–potassium carbonate method, the *p*-benzyl ether of this ester was obtained as an oil, forming a 2 : 4-*dinitrophenylhydrazone* which crystallised from ethyl acetate in scarlet needles, m. p. 226—227° (Found: C, 59·6; H, 4·5; N, 12·1. $C_{23}H_{20}O_7N_4$ requires C, 59·5; H, 4·3; N, 12·1%). On hydrolysis with 5% aqueous alcoholic potassium hydroxide this compound gave p-*benzyloxyphenylpyruvic acid* which was isolated with ether and purified by crystallisation from 50% acetic acid, forming colourless prisms, m. p. 192° after sintering at 180—182° (Found: C, 70·9; H, 5·3. $C_{16}H_{14}O_4$ requires C, 71·1; H, 5·2%). This compound had a green ferric reaction in alcohol.

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