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THE STRUCTURE OF PENDULIN AND PENDULETIN: A NEW FLAVONOL GLUCOSIDE ISOLATED FROM *BRICKELIA PENDULA*

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Abstract—The structure of a new flavone called penduletin (II), isolated as its glucoside (pendulin I) from *Brickelia pendula*, was established to be the 4′,5-dihydroxy-3,6,7-trimethoxy flavone. In pendulin, a glucose molecule is attached at the 4′ position.

WE have been interested for some time in the study of Mexican plants to which medicinal properties have been attributed. Among them are several species of plants known as *Atanasia amarga* that have a bitter taste and which are used as remedies for "stomach ailments". In a preliminary study carried out by Río de la Loza[1] it was reported that one of these *Atanasia amarga* (*Brickelia squarrosa*) contained a white glycoside, which was called "Brickelina".

We have found one of these species (which was classified as *Brickelia pendula**) in Contreras, near Mexico City.

Exhaustive extraction of the dried plant with alcohol yielded a green mass which on recrystallization from ethanol furnished a yellow crystalline substance which we have called *pendulin* (I), m.p. 178–179°; $[\alpha]_D^{20°}$ — 34° (pyridine) and empirical formula $C_{24}H_{26}O_{12}$. The infrared (Fig. 1), showed bands at 3200 (associated alcohol); 1660 (α-β unsaturated carbonyl); 1600 (aromatic ring); 1300–1180 [complex structure (multiple bands of medium intensity) due to absorption by the carbonyl and the methoxyls]; 1090 (alcoholic OH) and 838, 794 and 810 cm^{-1} (polysubstituted aromatic rings, which correspond to hydroxyl, methoxyl, ketone and phenyl groupings). λ_{max} 212, 272, 332 mμ; ε, 38,166; 23,013; 22,040.

Paper chromatography of this substance showed only one spot supporting the view that it is a pure substance.

From its empirical formula, physical constants and the different colors which it produced with specific reagents we came to the conclusion that the substance has a flavonoid structure monosubstituted with a carbohydrate.

It is difficult to hydrolyze pendulin but by prolonged boiling with strong acid a new substance was obtained which we have called penduletin (II). Its empirical formula is $C_{18}H_{16}O_7$; m.p. 216–217°. The infrared (Fig. 2) showed bands at 3100 (alcohol); 1660 (carbonyl); 1300–1180 (methoxyls) and 762 cm^{-1} (new band); λ_{max} 212, 271, 341 mμ; ε, 33,376; 19,231; 22,767. The substance has three methoxyl groups and forms a diacetate $C_{22}H_{20}O_9$ which on saponification regenerates penduletin (II). By methylation a pentamethoxy substance ($C_{20}H_{20}O_7$) (III) is obtained, while on ethylation a new compound ($C_{20}H_{20}O_7$) (IV) is formed corresponding to a monohydroxy-, mono-ethoxy-, trimethoxy-flavonoid. The sugar moiety was identified by paper chromatography and by its osazone as glucose. From the analysis before and

* We wish to thank Dr. F. Miranda of the Instituto de Biología of the Universidad Nacional Autónoma de México for his help in finding this plant and botanically classifying it.

[1] F. Río de la Loza *Materia Médica Mexicana.* Part I, p. 269 (1894).

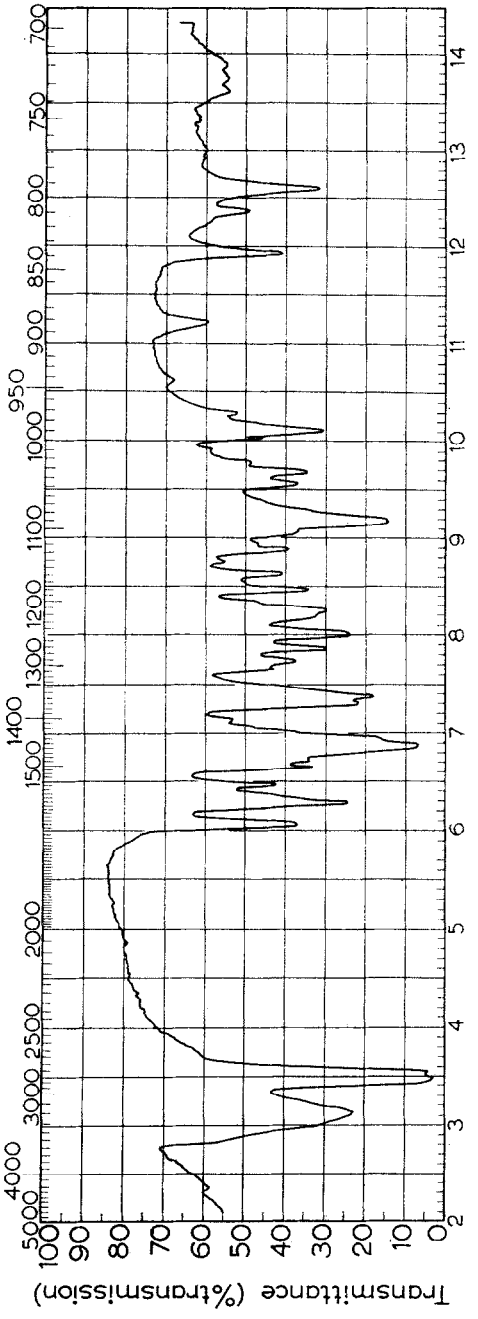


FIG. 1. Pendulin (I).

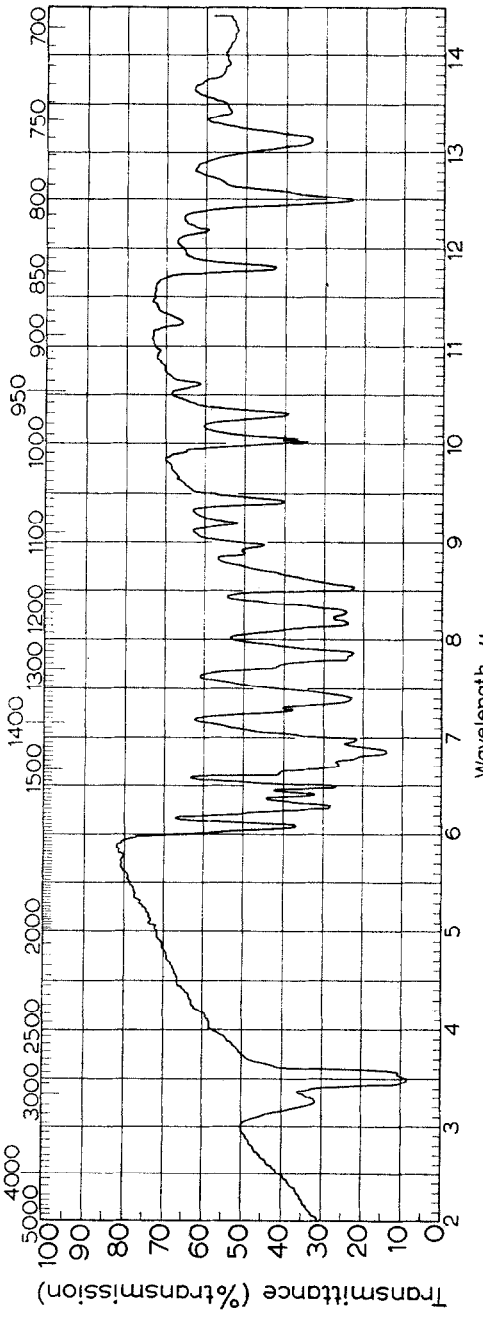


FIG. 2. Penduletin (II).

after hydrolysis it follows that pendulin is a trimethoxy-dihydroxy-flavonol or flavone substituted with one molecule of glucose.

By demethylation of compounds (II), (III) and (IV) a pentahydroxy compound (V) $C_{15}H_{10}O_7$ is obtained which by methylation regenerates the pentamethoxy flavone (III), demonstrating that demethylation does not produce rearrangement.[2] To prove that acid hydrolysis of pendulin does not produce rearrangement, it was methylated, hydrolyzed and remethylated, and gave (III).

When (III) was fused with potassium hydroxide it was completely destroyed, but by fission in alkaline solution it was possible to isolate *p*-methoxybenzoic acid. By ozonolysis of (III) we obtained the same acid exclusively. These facts demonstrate that the phenyl ring *B* of penduletin contains only one substituent, hydroxyl or methoxyl, present at the 4'position.

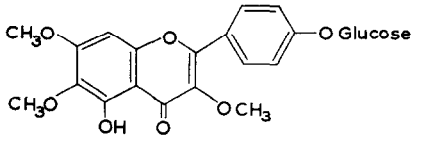
Compound (V) gives a series of color reactions identical to those reported by Goldsworthy and Robinson[3] for a synthetic 3,4',5,6,7-pentahydroxy-flavonol, the pentamethyl ether of which was supposed to be identical with tangeretin isolated by Nelson.[4] Recently Robinson has published a note[5] in which he proves that this synthetic compound is different from tangeretin. A sample of the pentamethyl ether of the synthetic compound of Goldsworthy and Robinson* showed the same infrared spectrum as our compound (III) and did not depress its melting point.

From this comparison, it follows that the five methoxyl groups of (III) are at positions 3,4',5,6 and 7. In penduletin three of the substituents occur as methoxyls and two as free hydroxyls.

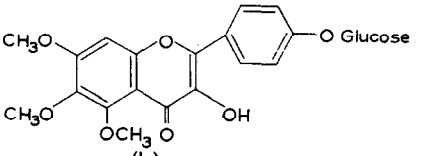
To determine the position of the glucosidic linkage pendulin (I) was methylated with diazomethane giving a tetramethoxy compound $C_{25}H_{28}O_{12}$ (VI) slightly more water soluble and with a bitter taste. By acid hydrolysis of (VI) we obtained a monohydroxy-tetramethoxy compound (VII) $C_{19}H_{18}O_7$.

By acetylation of (VII) the monoacetate (VIII) was obtained, which on ozonolysis gave *p*-acetoxybenzoic acid proving that the glucose in pendulin (I) is attached to the hydroxyl in position 4'.

There are, therefore, only two possible structures for pendulin: it must be either the 4'-glucoside of 4',5-dihydroxy-3,6,7-trimethoxyflavone (I) or the 4'-glucoside of 3,4'dihydroxy-5,6,7-trimethoxyflavone (Ia).



(I)



(Ia)

A decision in favor of (I) can be reached on the following grounds: (a) Ethylation of penduletin (II) with excess diethylsulfate and alkali introduces only *one* ethyl group, forming the 4'-ethyl derivative (IV). It is well known that ethylation of a 3-hydroxyl group proceeds readily under these conditions (cf. the ethylation of quercetin and morin[6]). On the other hand, survival of a 5-hydroxyl group under similar conditions

* We wish to thank Sir Robert Robinson for supplying us with this compound.

[2] S. K. Mukerjee and T. R. Seshadri *Chem. & Ind. (Rev.)* 271 (1955).

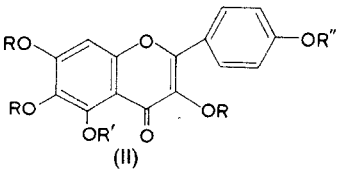
[3] L. J. Goldsworthy and R. Robinson *J. Chem. Soc.* 46 (1937).

[4] E. K. Nelson *J. Amer. Chem. Soc.* **56**, 1392 (1934).

[5] L. J. Goldsworthy and R. Robinson *Chem. & Ind. (Rev.)* 47 (1957).

is well documented.[6] (b) Treatment of the 4′-ethylether (IV) with acetic anhydride and sodium acetate in the cold produced no acetylation of the remaining free hydroxyl group. The 3-hydroxyl group of e.g. quercetin is easily acetylated under these conditions while the 5-hydroxyl is not touched.[7] (c) Finally, it is in keeping with the presence of the 5-hydroxylflavone system that the boric acid color test described by Wilson is positive, whereas a 3-hydroxyflavone without a 5-hydroxyl group, such as fisetin, gives a negative test.[8]

We conclude that pendulin and penduletin are correctly represented by (I) and (II), respectively.



(II)

(II) $R = CH_3$; $R' = R'' = H$

(III) $R = R' = R'' = CH_3$

(IV) $R = CH_3$; $R' = H$; $R'' = CH_3—CH_2—$

(V) $R = R' = R'' = H$

(VI) $R = R' = CH_3$; R'' = glucose

(VII) $R = R' = CH_3$; $R'' = H$

(VIII) $R = R' = CH_3$; $R'' = OAc$

EXPERIMENTAL*

Isolation of pendulin (I)

The sun dried plant (2·8 kg) was finely ground in a mikro-pulverizer mill and was extracted with three 10 l. portions of boiling alcohol. The combined extracts were concentrated under vacuum to a volume of 3 l. giving a very viscous green mass which was washed 5 times with 2 l. of hot hexane. The residue was a yellow powder, which was dissolved in alcohol, decolorized with Norite, recrystallized several times from alcohol and then from acetone–hexane from which 30 g of the glucoside *pendulin* (I) was obtained with m.p. 178–179°; $[\alpha]_D^{22}$ — 34° (pyridine), λ_{max} 212, 272, 332 mμ; ε, 38,166; 23,013; 22,040.

Anal. Calcd. for $C_{24}H_{26}O_{12}$: C, 56·91; H, 5·17; O, 37·91. Found: 57·13; H, 5·28; O, 37·72.

Calcd. for 3 methoxyls: 18·4. Found: 17·97.

* The microanalyses were carried out by Dr. Franz Pascher, Bonn, Germany. Absorptions in the ultraviolet were measured in ethanolic solution in a Beckman DK2 instrument. Infrared determinations were carried out on the Perkin-Elmer double beam model 21C spectrophotometer and were done in Nujol. Melting points are uncorrected and were determined in a Kofler block.

[6] A. G. Perkin and S. Phipps *J. Chem. Soc.* **85,** 56 (1904); J. Herzig *Mh. chem.* **5,** 72 (1884); **9,** 537 (1888); A. S. Gomm and M. Nierenstein *J. Amer. Chem. Soc.* **53,** 4408 (1931); A. G. Perkin *J. Chem. Soc.* **103,** 209 (1913).

[7] O. Kubota and A. G. Perkin *J. Chem. Soc.* **127,** 1889 (1925).

[8] C. W. Wilson *J. Amer. Chem. Soc.* **61,** 2303 (1939).

[9] T. B. Gage, C. D. Douglas and S. H. Wender *Analyt. Chem.* **23,** 1582 (1951).

In order to test the purity of the glucoside a paper chromatogram was carried out on Whatman No. 1 filter paper using *n*-butyl alcohol–acetic acid–water 40/10/50 at 23° and developing over 8 hr. The dried paper was observed in ultraviolet and visible light. This showed only one spot with $R_f = 0{\cdot}76$.[9]*

Hydrolysis of the glucoside (I)

(I) After attempts to hydrolyze (I) with 5 per cent hydrochloric acid the glucoside was recovered unchanged. It was then hydrolyzed by dissolving 0·5 g in 300 ml of aqueous alcohol (70 per cent) containing 15 per cent of HCl. The mixture was boiled for 6 hr and was then diluted with 1 l. of water and extracted 4 times with 200 ml of ethyl acetate. The combined extracts were dried with sodium sulfate and evaporated to dryness. The residue was crystallized from alcohol–water and then from acetone–water thus producing 0·3 g of pale yellow *penduletin* (II) m.p. 216–217°; λ_{max} 212, 271, 341 mμ; ε, 33,376; 19,231; 22,767.

Anal. Calcd. for $C_{18}H_{16}O_7$: C, 62·79; H, 4·68; O, 32·53. Found: 62·98; H, 4·76; O, 32·53.

Calcd. for 3 methoxyls: 27·03. Found: 26·05.

Identification of the carbohydrate

The aqueous fraction from the acid hydrolysis was concentrated under vacuum to a volume of 500 ml. After neutralization, the liquid gave positive reactions with Fehling and Tollens reagents.

A parallel paper chromatogram was carried out with a small fraction of the aqueous extract and with a solution of glucose, developing according to the Horrocks and Manning technique.[11] The two spots thus obtained were identical.

Microscopic observation of the osazone[12] and its melting point showed that it was glucosazone. A parallel paper chromatogram of this product and of an authentic sample of glucosazone, following a technique similar to that reported by Burton, Zaffaroni and Keutmann[13] and used by Muñoz,[14]† confirmed it.

Color reactions of penduletin (II)

By treating the alcoholic solution of penduletin with sodium amalgam a crimson coloration was obtained. On treating the original solution with hydrochloric acid and magnesium a pink color was obtained.[15] Penduletin in concentrated sulfuric acid solution gave an intense yellow color. The color reactions point to a flavonol.[16]

Acetylation of penduletin (II)

Penduletin (0·5 g) was dissolved in acetic anhydride (60 ml), 2 drops of perchloric acid were added and the solution allowed to remain at room temperature for 40 min. It was then diluted with 500 ml of water and kept for 6 hr. The solution was then

* We wish to thank Dr. Barbarín Arreguín of this Institute for carrying out this experiment.

† E. Muñoz used, in the separation of osazones, Whatman No. 1 filter paper previously washed with water and ethanol, using as mobile phase chloroform saturated with formamide and as stationary phase formamide-methanol in equal parts, by volume.

[11] R. H. Horrocks and G. B. Manning *Lancet* **256,** 1042 (1949).

[12] W. Z. Hassid and R. M. McCready *Industr. Engng. Chem. (Anal.)* **14,** 683 (1942).

[13] R. B. Burton, A. Zaffaroni and E. H. Keutmann *J. Biol. Chem.* **188,** 763 (1951).

[14] E. Muñoz, Thesis Separación identificación y determinación cuantitativa de algunas osazonas por cromatografía en papel. Instituto Politécnico Nacional, México (1955).

[15] W. K. Warburton *Quart. Reviews* **VIII,** 70 (1954).

[16] T. A. Geissman *Moderne Methoden der Planzenanalyse* (Edited by K. Paech and M. V. Tracey) Vol. III, p. 470 (1955).

extracted with 3 × 50 ml portions of ethyl acetate. The organic layer was washed with cold 3 per cent aqueous sodium hydroxide and then with distilled water until neutral and was then evaporated to dryness. The residue was crystallized from acetone–hexane and gave 0·4 g of the acetylated product, m.p. 157–158°.

Anal. Calcd. for $C_{22}H_{20}O_9$: C, 61·68; H, 4·71; O, 33·61. Found: C, 62·00; H, 4·88; O, 33·27.

Calcd. for 2 acetyls: 20·00 Found: 21·96.

Saponification of diacetyl penduletin

The diacetate (0·1 g) was saponified by boiling it for 1 hr with alcoholic sodium hydroxide. The solution was processed as usual. The product (0·07 g) had m.p. 216–217° and the mixed m.p. with (II) gave no depression. The infrared spectra were also identical.

Methylation of penduletin (II)

Penduletin (0·5 g) was methylated with diazomethane using the usual technique. A white product (III) (0·35 g) was obtained, which after crystallization from ethanol and then from acetone–hexane showed m.p. 152–153°.

Anal. Calcd. for $C_{20}H_{20}O_7$: C, 64·51; H, 5·41; O, 30·08. Found: C, 64·80; H, 5·22; O, 30·07.

Calcd. for 5 methoxyls: 41·60 per cent. Found: 40·88 per cent.

Monoethylation of penduletin (II)

Penduletin (0·5 g) was dissolved in 100 ml of ethanol to which 20 ml of 20 per cent sodium hydroxide in 70 per cent aqueous ethanol solution was added. Then 10 ml of diethyl sulfate was slowly added, the mixture was boiled for 30 min and an additional 20 ml of diethyl sulfate was added. The solution was boiled for another 5 hr, keeping it alkaline all the time. After cooling it was diluted with 300 ml of water and extracted with ethyl acetate. Evaporation of the solvent and crystallization of the residue from ethanol–water and from acetone–hexane gave 0·31 g of compound (IV), with m.p. 166–167°; λ_{max} 212, 272, 336 mμ; ε, 39,690; 22,138; 24,711.

Anal. Calcd. for $C_{20}H_{20}O_7$: C, 64·51; H, 5·41; O, 30·08. Found: C, 64·79; H, 5·44; O, 29·99.

Calcd. for 4 methoxyls (one ethoxyl as methoxyl) 33·33. Found: 32·22.

Calcd. indirectly for 3 methoxyls: 25·00. 1 ethoxyl: 12·10. Found: 24·14. 11·73.

The acetylation of IV was done as in the case of penduletin (II) and the acetate had m.p. 149–150°.

Demethylation of penduletin (II)

Demethylation in the usual manner with 47 per cent hydriodic acid and acetic anhydride, gave a product that still contained one methoxyl m.p. 286–289° (d).

Anal. Calcd. for $C_{16}H_{12}O_7$: C, 60·76; H, 3·82. Found: 60·94; H, 4·01.

Calcd. for one methoxyl: 9·80. Found: 9·07.

Demethylation under more energetic conditions leads to the pentahydroxy flavonol. To a solution of 0·4 g of penduletin (II) in 10 ml of propionic anhydride, 0·3 g of phenol and 18 ml of freshly distilled 55 per cent hydriodic acid were added. The mixture was boiled for 2 hr and was then diluted with 100 ml of water. It was

extracted with ether and with ethyl acetate. The bulked organic fractions were washed three times with an aqueous solution of sodium thiosulfate to eliminate the iodine liberated during the reaction. After drying and evaporating the solvents the residue was crystallized twice from acetone–hexane from which 0·15 g of (V) was obtained. The melting point is not definite and the substance begins to decompose at 290° and chars between 314 and 320°.

Anal. Calcd. for $C_{15}H_{10}O_7$: C, 59·61: H, 3·34; O, 37·06. Found: C, 59·01; H, 3·59; O, 37·20.

By acetylation of (V) the penta-acetate is obtained, m.p. 234–235°.

By methylation with diazomethane by the usual technique (V) regenerates (III) proving that no rearrangement occurred during the demethylation.

Alkaline fission

Half a gram of (III) was dissolved in 50 ml of ethanol and 50 ml of 20 per cent potassium hydroxide in water–alcohol was added. The mixture was boiled for 20 hr, then concentrated, diluted with water and saturated with carbon dioxide. By extraction with ether no phenolic fraction was obtained. The aqueous solution was acidified with hydrochloric acid and extracted with ether. The ethereal fractions were evaporated under vacuum and the residue was recrystallized from acetone–water when 0·05 g of an acid with m.p. 183–184° was obtained. The mixed melting point with an authentic sample of *p*-methoxy-benzoic acid was not depressed and the infrared spectra were identical. A similar fission with (IV), gave an acid which showed m.p. 194–195°. The m.p. was not depressed when mixed with an authentic sample of *p*-ethoxybenzoic acid and the infrared spectra were the same.

Ozonolysis of penduletin (II)

A solution of 0·1 g of the diacetate of penduletin in 40 ml of anhydrous ethyl acetate was ozonized for 7 min in a T23 Welsbach ozonator with 0·02 flow of oxygen at a pressure of 8 lb and 90 V. The ozonide was decomposed by catalytic hydrogenation using 5 per cent palladium on charcoal. The amount of hydrogen absorbed was 150 ml. The catalyst was filtered and the residue after removal of the solvent was crystallized from acetone–benzene and sublimed under high vacuum (0·01 mm and 125°). An acid (0·02 g) was obtained of m.p. 188–190°; the mixed melting point with *p*-acetoxybenzoic acid was not depressed and the infrared spectra were identical.

This method gives much better yields than the alkaline fission and might be used advantageously in similar cases.

Color reactions of the pentahydroxy flavonol (V)

With hydrochloric acid and magnesium a pink color was obtained; with lead acetate in alcoholic solution an orange precipitate was formed that later turned brown. With ferric chloride a dark olive green was obtained. These reactions were exactly like those reported by Robinson[3] for 3,4′,5,6,7,-pentahydroxy-flavonol.

The position of the 5 hydroxyls was proved by comparison of the dimethoxy-penduletin (III) with 3,4′,5,6,7-pentamethoxy-flavone kindly supplied by Sir Robert Robinson. The mixed melting point was not depressed and the infrared spectra were exactly alike.

Methylation of pendulin (I)

It is difficult to methylate the glucoside with diazomethane under normal conditions. In order to methylate it an ethereal solution of diazomethane, obtained from 18 g of nitrosomethyl urea, was added to 1 g of pendulin dissolved in methanol and the solution was allowed to stand for 24 hr. At that time a fresh solution of diazomethane, obtained from 10 g of nitrosomethyl urea was added, and this treatment was repeated after 24 hours. At the end of the third 24 hr period the solution was evaporated under vacuum without applying heat. The dry residue was dissolved in anhydrous acetone and chromatographed on "Magnesol" (150 g). The column was eluted first with acetone, then with ethyl acetate saturated with water and then with 50 per cent aqueous ethanol.[17] The desired product was obtained from this last solvent after evaporation to dryness, crystallization from alcohol–water and from acetone–water, all in a nitrogen atmosphere. A water soluble compound (VI) (0·6 g) was obtained which had a bitter taste and melted at 227–228° (d) λ_{max} 212, 267, 326 mμ; ε, 39, 651; 27, 627; 29, 695.

Anal. Calcd. for $C_{25}H_{28}O_{12}$: C, 57·69; H, 5·42; O, 36·89. Found: 57·58; H, 5·63; O, 36·94.

Calcd. for 4 methoxyls: 23·85. Found: 23·82.

Hydrolysis of methoxy pendulin (VI)

A solution of 0·8 g of (VI) in 50 ml of a 10 per cent aqueous ethanolic solution of hydrochloric acid was boiled for 3·5 hr. It was diluted with 100 ml of water and extracted 3 times with 40 ml of ethyl acetate. After the usual work-up the product was crystallized from ethanol–water giving 0·4 g of (VII) m.p. 253–254°. λ_{max} 212, 260, 330 mμ; ε, 38,871; 19,630; 26,641.

Anal. Calcd. for $C_{19}H_{18}O_7$: C, 63·68; H, 5·06; O, 31·25. Found: 64·06; H, 4·84; O, 31·21

Calcd. for 4 methoxyls: 34·63. Found: 34·64.

Methylation of (VII)

Compound (VII) (0·2 g) was methylated by the usual technique with diazomethane giving 0·12 g of a compound which proved to be the same as (III) by mixed melting point and infrared spectra.

By acetylation of (VII) (0·15 g) carried out as before a mono-acetate (VIII) was obtained (0·08 g) m.p. 151–153° (d).

Ozonolysis of the acetate (VIII)

The monoacetate (VIII) (0·075 g) was dissolved in anhydrous ethyl acetate and ozonized, as in the previous case, for 3 min. The ozonide was hydrogenated with 0·1 g of 5 per cent palladium on charcoal. It absorbed 100 ml of hydrogen. The catalyst was filtered off and the solvent evaporated. The residue was crystallized from acetone–benzene and sublimed under high vacuum (0·01 mm and 125°). A product (0·013 g) was obtained which melted at 188–190° and the m.p. was undepressed when the substance was mixed with an authentic sample of *p*-acetoxybenzoic acid. The infrared spectra of the samples were identical. It is therefore concluded that the glucose molecule is attached to the 4′ position of penduletin (II).

[17] C. H. Ice and S. H. Wender *Analyt. Chem.* **24**, 1616 (1952).