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CHEMICAL EXAMINATION OF ANDROGRAPHIS ECHIOIDES—II[1]

STRUCTURE AND SYNTHESIS OF ECHIOIDIN

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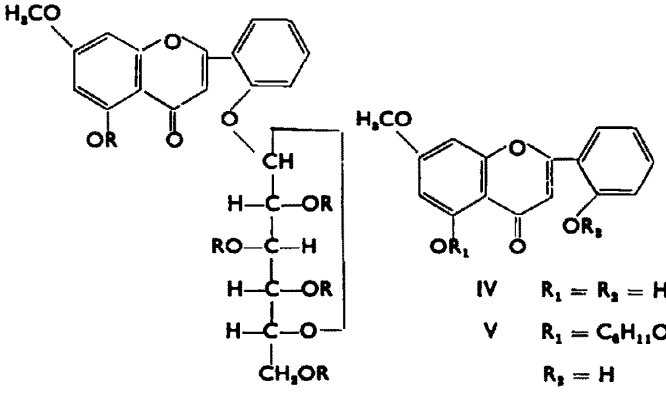
Abstract—On the basis of degradative and spectral evidence and synthesis, echioidin, the new flavone glucoside isolated from *Andrographis echioides* Nees, is shown to be 5-hydroxy-2′-β-D-glucosidoxy-7-methoxyflavone (echioidinin-2′-β-D-glucoside).

RECENTLY, we reported[1] the isolation of a new flavone, echioidinin, and a flavone glucoside, echioidin, from the acetone extracts of *Andrographis echioides* Nees. By a combination of degradative and spectral evidence and synthesis echioidinin was shown to be 5,2′-dihydroxy-7-methoxy-flavone[1] (IV). The present paper deals with the structure and synthesis of the glucoside, echioidin (I).

Echioidin (see Experimental), analyses for the formula $C_{22}H_{22}O_{10}$ and contains one methoxyl group. Echioidin dissolves easily in dilute alkali giving a yellow solution. It produces a brown colour with alcoholic ferric chloride and gives a positive Molisch test.

The UV spectrum of echioidin (Fig. I) is similar to that of echioidinin[1] and the IR spectrum (Fig. II) exhibits sharp bands at 3540, 3360 cm^{-1} (OH), 1650 cm^{-1} (C=O), 1600, 1575 and 1500 cm^{-1} (aromatic) reminiscent of a flavonoid compound.

Alkaline hydrogen peroxide oxidation of echioidin gives salicylic acid and a phenol m.p. 261–263° which from its physical properties was identified as echioidinin (IV). Fission of echioidin with 50% aqueous potassium hydroxide, on the other



I R = H
II R = AC
III R = CH_3

IV $R_1 = R_2 = H$
V $R_1 = C_6H_{11}O_5$, $R_2 = H$
VI $R_1 = H$; $R_2 = C_6H_{11}O_5$
VII $R_1 = CH_3$; $R_2 = H$
VIII $R_1 = CH_3$; $R_2 = C_2H_5$

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[1] Part I, T. R. Govindachari, P. C. Parthasarathy, B. R. Pai and P. S. Subramaniam, *Tetrahedron* **21**, 2633 (1965).

hand, affords a mixture of five products, three of which were identified as phloroglucinol monomethyl ether, echioidinin (IV) and salicylic acid by thin layer chromatography.

Acetylation of echioidin gives a crystalline penta-acetyl derivative (II) (see Experimental) which on hydrolysis with 10% alcoholic hydrochloric acid does not regenerate echioidin (I) but yields echioidinin (IV) quantitatively. Furthermore, drastic hydrolysis of echioidin with 20% sulphuric acid gives echioidinin (IV) and D-glucose.

From the foregoing facts it is evident that echioidin may be formulated as a glucoside of echioidinin. Of the two possible structures (V and VI) V was ruled out since echioidin gives a positive ferric reaction and resists methylation with diazomethane indicating the presence of a 5-hydroxyl group.[2] This conclusion was confirmed by the spectral shifts observed on the addition of aluminium chloride and sodium ethoxide (Fig. 1). The aluminium complex spectrum is characteristic of a 5-hydroxyflavone, both bands I and II undergoing bathochromic shifts.[3] The sodium ethoxide spectrum is typical of a partially glycosidated flavone whose only free hydroxyl is located in the 5-position;[4,5] the short wavelength band is shifted towards longer wavelength by 12 mμ and the long wavelength band becomes merely an inflexion of very low intensity.

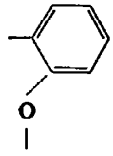
The NMR spectrum of echioidin acetate (Fig. III) is in complete accord with structure I. The integrated spectrum shows a total of 32 protons; the chemical shifts and the probable assignments are given in Table 1. The aromatic acetate methyl signal at 2·43δ is in the correct position for the 5-acetate group since this group in a flavone absorbs near 2·46δ, distinct from other acetate groups which absorb near 2·34δ.[6]

Unambiguous proof for the point of attachment of the sugar residue in echioidin was obtained by the standard method of methylation, acid hydrolysis and identification of the hydroxymethoxy compound.[7] Echioidin can not be methylated by the usual dimethyl sulphate–acetone–potassium carbonate method owing to the very sparing solubility of the compound in acetone, but it is readily methylated by Kuhn's method[8] with dimethyl sulphate, barium oxide and barium hydroxide in dimethyl sulfoxide and dimethylformamide. The permethyl derivative (III), whose NMR spectrum indicates the presence of four aliphatic and two aromatic methoxyl groups, on hydrolysis with 10% sulphuric acid gives an aglycone identical with an authentic specimen of 5,7-dimethoxy-2′-hydroxyflavone[1] (VII). The identity was further established through their respective ethyl ethers (VIII), thus proving that the glucose moiety in echioidin is attached at 2′-position. The methylated sugar residue in the above hydrolysis was identified as 2,3,4,6-tetramethylglucose by paper chromatography.

Finally structure I for echioidin has been confirmed by synthesis: Partial glucosidation of echioidinin (IV) with one mole of α-bromo-tetra-acetylglucose according

[1] L. H. Briggs and R. H. Locker, *J. Chem. Soc.* 3136 (1951).
[2] L. Jurd in *The Chemistry of Flavonoid Compounds* (Edited by T. A. Geissman) p. 119. Pergamon Press, London (1962).
[3] Ref. 3, p. 124.
[4] G. H. Mansfield, T. Swain and C. G. Nordstrom, *Nature, Lond.* 172, 23 (1953).
[5] C. A. Henrick and P. R. Jefferies, *Austr. J. Chem.* 17, 934 (1964).
[6] K. Venkataraman, Ref. 3 p. 99.
[7] R. Kuhn and H. Trischmann, *Chem. Ber.* 96, 284 (1963); and Refs cited therein.

TABLE 1. NMR SPECTRUM OF ECHIOIDIN ACETATE

Chemical shifts (δ values)	Multiplicity (J values)	Proton intensity	Assignments of the signals
1·85, 1·97, 2·05 and 2·08	Singlet	12 (each 3 proton intensity)	Aliphatic acetates of the glucose moiety
2·43	Singlet	3	Aromatic acetate (5-position acetate)
3·95	Singlet	4	Aromatic methoxyl (7-position methoxyl) and H_{C-5} of the acetylated glucose[9]
4·27	Multiplet	2	HCH_2 of the acetylated glucose
5·15 — 5·35	Multiplet	4	Protons of the C-1, C-2, C-3 and C-4 of the acetylated glucose[9]
6·52	Singlet	1	Heterocyclic (C_3) proton
6·62	Doublet (J = 2·5 c/s)	1	C_6-proton
6·85	Doublet (J = 2·5 c/s)	1	C_8-proton
7·2 — 7·75	Multiplet	4	
Total number of protons	32	corresponding to $C_{33}H_{32}O_{15}$	

to the procedure of Zemplen and Farkas,[10] gives a product which is identical in all respects with natural echioidin.

A survey of the naturally occurring flavonoid glycosides reveals that echioidin is the first example of a naturally occurring flavone glucoside with the sugar moiety linked to the 2′-hydroxyl group.

EXPERIMENTAL

Mps. were determined in capillaries and are uncorrected. The UV spectra were measured in 95% EtOH using a Beckmann Model DU spectrophotometer. NMR spectra were taken in $CDCl_3$ on a 60 m.c. Varian instrument with TMS as an internal standard. Kieselgel G and aluminium oxide G (E. Merck) were used for TLC.

(i) *Echioidin.* Isolated as described earlier,[1] echioidin was purified by repeated crystallization from pyridine–MeOH. It formed clusters of colourless short needles, m.p. 276–78° (dec.), $[\alpha]_D^{26°}$ −23·5° (*c*, 0·213 in pyridine-MeOH, 1:1). It was very sparingly soluble in water and most of the common

[9] A. von Wartburg, M. Kuhn and H. Lichti, *Helv. Chim. Acta* **47**, 1203 (1964).
[10] G. Zemplen and L. Farkas, *Ber. Dtsch. Chem. Ges.* **76**, 1110 (1943).

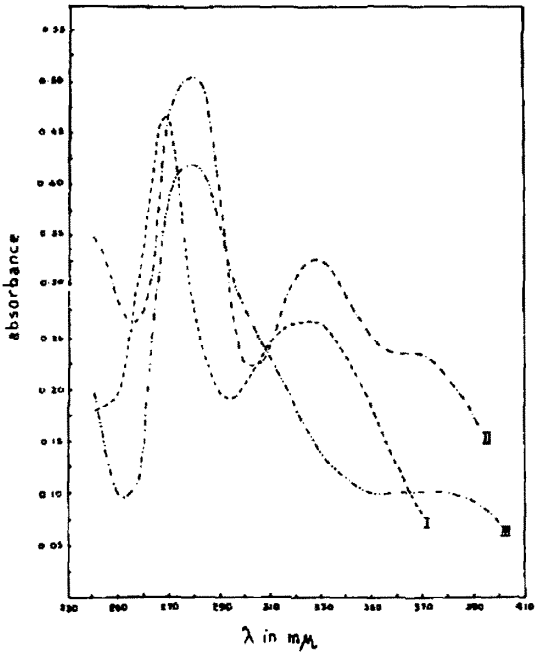


FIG. I UV spectrum of: (i) Echioidin in absolute ethanol, (ii) Echioidin in absolute ethanol plus aluminium chloride (iii) Echioidin in absolute ethanol plus sodium. ethoxide

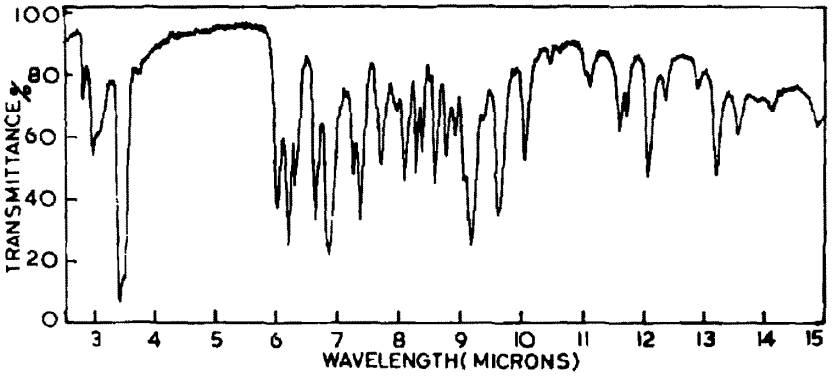


FIG. II IR Spectrum of Echioidin. (Nujol Mull) *Structure and Synthesis of Echioidin*

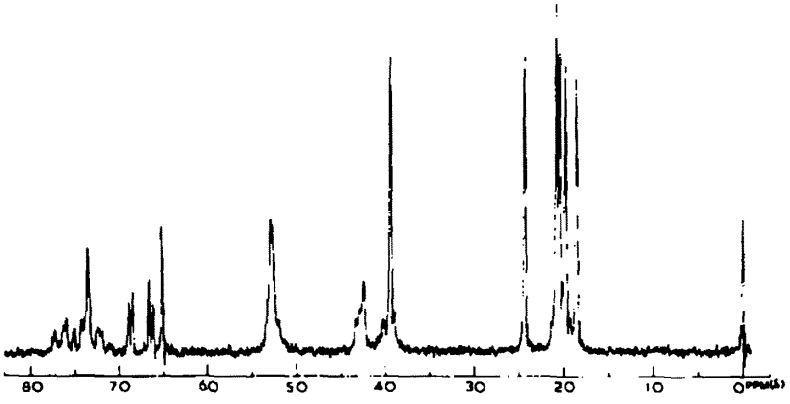


FIG. III NMR spectrum of Echioidin acetate in $CDCl_3$ (60 Mc/sec).

organic solvents and dissolved readily in dimethyl sulfoxide and hot pyridine. It dissolved easily in aqueous alkali giving a yellow solution. It gave a brown colour with alcoholic ferric chloride and no colour with Mg–HCl or Zn–HCl. The Wilson's boric acid test could not be carried out owing to the very sparing solubility of the compound in acetone. Echioidin gave a positive Gibbs test, with the indophenol chromophore absorbing at 660 mμ, and a positive Molisch test. UV* λ_{max}^{EtOH} 230–35 (sh), 268, 320–330 mμ; $\lambda_{max}^{EtOH-AlCl_3}$ 240, 280, 325–330, 360–370 (sh) mμ; $\lambda_{max}^{EtOH-NaOEt}$ 280, 350–370 (inflexion) mμ. (Found: C, 59·3; H, 5·1; OCH_3, 3·6. $C_{22}H_{22}O_{10}$ requires: C, 59·2; H, 4·9; 1 OCH_3, 3·4%).

(ii) *Alkaline hydrogen peroxide oxidation of echioidin.* Echioidin (0·25 g) in alcohol (6 ml) was refluxed gently with a solution of KOH (2 g) in water (6 ml) for 3 hr. After being cooled to 15°, the reaction mixture was treated dropwise with H_2O_2 (30%; 6 ml) and allowed to stand overnight at room temp. Next day the clear solution was evapotated to dryness *in vacuo* and the residue dissolved in water (8 ml), cooled and acidified. It was extracted exhaustively with ether and the ether solution treated with sat. $NaHCO_3$ aq (3 × 4 ml). The bicarbonate extract was acidified with cooling and re-extracted with ether. After being dried (Na_2SO_4), the ether was distilled off and the residue sublimed *in vacuo*. The colourless sublimate was crystallized from benzene–pet ether (b.p. 40–60°) giving colourless needles, m.p. 156–157° undepressed on admixture with authentic *salicylic acid*. The IR spectra were identical.

The ether solution left after extraction with bicarbonate was washed with water, dried (Na_2SO_4) and ether distilled off. A pale yellow residue was obtained which after two crystallizations from alcohol melted at 261–263° alone or when mixed with *echioidinin*.

(iii) *Alkali fission of echioidin.* Echioidin (50 mg) was refluxed gently in an oil-bath (160–165°) with 50% KOH aq (3 ml) for 30 min. The resulting clear yellow solution was cooled, diluted with water (2 ml) and acidified with conc. HCl. It was extracted with ether exhaustively (5 × 20 ml) and the ether solution treated with sat. $NaHCO_3$ aq (2 ml). The bicarbonate solution, after acidification, was thoroughly extracted with ether (5 × 10 ml), the ether solution dried (Na_2SO_4) and evaporated. The residue was identified as salicylic acid by TLC (silica; benzene: MeOH: n-butyl acetate—20:6:6); authentic salicylic acid run side by side gave an identical spot. The ether solution left after extraction with bicarbonate was evaporated and the gummy residue examined by TLC (silica) (benzene: MeOH:n-butyl acetate–20:4:1); four spots were revealed of which two (the major spots) corresponded exactly with the spots of phloroglucinol monomethyl ether and echioidinin which were run as standards.

(iv) *Echioidin acetate.* Echioidin (0·3 g), anhydrous pyridine (4 ml) and acetic anhydride (4 ml) were heated on a steam-bath for 2 hr, cooled and poured into ice-water. The resulting precipitate was collected, washed with water, dried (0·4 g) and recrystallized from alcohol giving the *acetyl derivative* (0·32 g) as colourless needles, m.p. 192–193° $[\alpha]_D^{34°}$ –72·85° (*c*, 1·57 in acetone). It gave a yellow colour with both Mg-HCl and Zn–HCl and no colour with $FeCl_3$. UV: λ_{max}^{EtOH} 245-250 (sh), 303 mμ (log *e* 4·37, 4·32). (Found: C, 58·4; H, 4·7; OCH_3, 2·4. $C_{32}H_{28}O_{16}$ requires: C, 58·5; H, 4·9; 1 OCH_3; 2·3%).

(v) *Hydrolysis of echioidin acetate.* A mixture of echioidin acetate (0·15 g) and 10% alcoholic HCl (16 ml) was heated on the steam-bath for 2 hr. The precipitated crystalline yellow solid was collected, washed, dried (0·75 mg) and recrystallized from alcohol giving *echioidinin* as pale yellow needles, m.p. and mixed m.p. 261–263°.

(vi) †*Acid hydrolysis of echioidin.* (Identification of echioidinin and D-glucose): A mixture of echioidin (0·3 g) and 20% H_2SO_4 (50 ml) was heated under reflux with vigorous stirring for 30 hr. After being cooled the resulting precipitate was collected, dried and crystallized twice from EtOH, giving the aglycone as greenish-yellow needles, m.p. 261–63° (dec) alone or when mixed with *echioidinin*. The identity was further confirmed by TLC and comparison of their acetyl derivatives (m.p., m.m.p. and IR).

The aqueous acid filtrate, obtained above, was neutralized by gradual addition of $BaCO_3$ with stirring. The precipitated $BaSO_4$ was filtrated off, washed well with water and the combined filtrates were concentrated to a small volume (~5 ml) *in vacuo*. A small amount of norite was added and the mixture filtered. The clear filtrate was evaporated to dryness *in vacuo*, the syrupy residue dissolved in water (2 ml) and subjected to paper chromatography. A single spot, corresponding exactly with

* UV qualitative owing to the very sparing solubility in EtOH.

† Unlike the normal flavone glycosides, echioidin was unusually very resistant to acid hydrolysis and required rather drastic conditions for complete hydrolysis.

that of D-*glucose* run as standard side by side, was revealed in different solvent systems: (a) butan-1-ol: EtOH:water—3:1:1; (b) ethyl acetate:pyridine:water—10:4:3; (c) butan-1-ol:EtOH:water—5:1:4. The presence of glucose was further confirmed by preparing the phenylosazone by the usual method to prove the identity with phenylglucosazone, m.p. and m.m.p. 205–207°.

(vii) *Methylation of echioidin.* To a solution of echioidin (0·4 g) in dimethyl sulfoxide (10 ml) and dimethyl-formamide (10 ml) was added, with ice cooling, BaO (3 g) and $Ba(OH)_2$ (3 g). Dimethyl sulphate (8 ml) was then added with vigorous stirring during 5 min, ice cooling being maintained till the addition was over. The mixture was stirred at room temp for 24 hr. At the end ammonia liquor (4 ml) was added and stirring continued for a further period of 30 min, to decompose excess of dimethyl sulphate. The viscous product so obtained was digested with $CHCl_3$ (3 × 50 ml) and the extract washed with water, dried (Na_2SO_4) and the solvent distilled off. Removal of the residual dimethyl sulfoxide *in vacuo* at steam bath temp gave, on cooling and trituration with pet. ether (b.p. 40–60°), a colourless amorphous solid (0·32 g), a TLC (alumina) (ethyl acetate or benzene: MeOH—25:1) which showed a major spot and in addition three other less intense spots. This crude methylated product was then dissolved in benzene and chromatographed over alumina (28 g), in the same solvent. The benzene eluate gave only oily material. Elution with ethyl acetate (10 ml fractions) gave the desired product in the first three fractions which were combined and crystallized repeatedly from ethyl acetate–pet. ether (b.p. 40–60°) giving colourless needles of *permethylechioidin* (0·22 g), m.p. 116–117° (single spot in TLC), $[\alpha]_D^{24°}$ −90·22° (*c*, 0·87 in acetone). UV: λ_{max}^{EtOH} 260, 310 mμ (log ε 4·41, 4·20). NMR: Signals at 3·4, 3·55, 3·66, 3·88 and 3·95 δ. (Found: C, 61·4; H, 6·5. $C_{27}H_{32}O_{10}\cdot\frac{1}{2}H_2O$ requires: C, 61·7; H, 6·3%).

(viii) *Hydrolysis of permethylechioidin.* Permethylechioidin (0·2 g) and 10% H_2SO_4 (50 ml) were heated under reflux (oil-bath, 155–160°) with vigorous stirring for 4 hr. After being cooled, the resulting colourless microcrystalline precipitate was collected, washed with water and dried (110 mg). It gave no colour with $FeCl_3$ and crystallization from EtOH gave colourless needles, m.p. 281–283° alone or when mixed with an authentic specimen of 5,7-*dimethoxy-2'-hydroxyflavone*.[1] The identity was further established by TLC and IR spectra. UV: λ_{max}^{EtOH} 260, 330–335 mμ (log ε 4·42, 4·25). (Found: C, 68·4; H, 4·81. $C_{17}H_{14}O_6$ requires: C, 68·5; H, 4·7%).

The aqueous acid filtrate from the aglycone, after being worked up in the usual way, was subjected to paper chromatography (butan-1-ol; EtOH:water—5:1:4). A single spot was obtained, identical with that of 2,3,4,6-*tetramethylglucose* run as standard.

(ix) 5,7-*Dimethoxy-2'-ethoxyflavone*. 5,7-Dimethoxy-2'-hydroxyflavone (0·1 g) obtained above, diethyl sulphate (1 ml), ignited K_2CO_3 (2 g) and acetone (50 ml) were refluxed for 12 hr. The reaction mixture was filtered hot, the potassium salts washed with hot acetone and the acetone distilled off. Water was added to the residue and the precipitate thus obtained extracted with $CHCl_3$. The washed, dried (Na_2SO_4) $CHCl_3$-solution afforded a greenish residue on removal of the solvent. It was dissolved in benzene and chromatographed over alumina. Elution with $CHCl_3$, followed by crystallization from benzene afforded colourless needles of 5,7-*dimethoxy-2'-ethoxyflavone* (60 mg), m.p. 165°. It was identical in all respects (mixed m.p. and IR) with a specimen prepared by ethylating authentic 5,7-dimethoxy-2'-hydroxyflavone.[1]

(x) *Synthesis of echioidin.* Echioidinin (0·7 g) was dissolved in 9% KOH aq (2·2 ml) and diluted with acetone (10 ml). A solution of acetobromoglucose (1·0 g) in acetone (10 ml) was then added dropwise with stirring, the temp being maintained at 20°. After being stirred at 20–25° for 12 hr, the mixture was poured into ice-water (200 ml) with stirring and the resulting precipitate collected, after leaving in an ice chest for 6 hr, and dried (1 g). It was treated with warm $CHCl_3$ (20 ml), the insoluble unreacted echioidinin (0·3 g) removed by filtration and the clear filtrate evaporated. The residue (0·6 g) was dissolved in alcohol (8 ml), treated with 3% NaOH aq (6 ml) and warmed for a few min on a water bath. It was then cooled and acidified with dil HCl. The pale yellow colloidal precipitate was collected, dried, refluxed with alcohol (25 ml) and filtered hot. The alcohol insoluble part (0·3 g) on crystallization from pyridine–MeOH gave a colourless powdery compound, m.p. 276–78° alone or when mixed with echioidin, $[\alpha]_D^{27·5°}$ −26·68° (*c*, 0·405 in pyridine–MeOH). The IR spectra in KBr were identical. (Found: C, 59·3; H, 5·0%).

The *acetyl derivative* prepared by the usual method, had m.p. and mixed m.p. with echioidin acetate 192–193°. The IR spectra in KBr were superposable. (Found: C, 58·8; H, 5·0%).

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