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THE SPECTROSCOPIC IDENTIFICATION OF CONIFERIN

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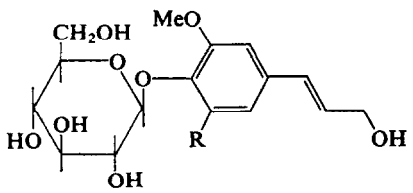
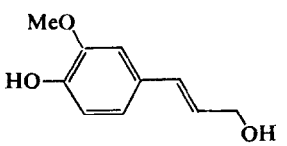
Abstract—A phenolic glycoside was isolated from *Balanophora polyandra* Griff. and identified as coniferin (I) by a combination of spectroscopic methods and mass spectrometry.

Balanophora polyandra Griff. grows in Thailand and Laos and it belongs to one of the lesser known families, Balanophoraceae. Examination of the whole aerial part of this plant by two of us (S. M. and V. P.) yielded a white, crystalline compound, m.p. 187°. The preliminary investigation of this compound suggested that it was probably a glycoside, but study by acid hydrolysis was not very rewarding. Physical methods, however, soon identified the compound as the known glycoside, coniferin (I), but as the results obtained by NMR spectroscopy and mass spectrometry have some features of general interest, a short summary of our work is reported.



Coniferin (I), R = H
Syringin (II), R = OMe



Coniferyl Alcohol (III)

Coniferin was first isolated from conifers (*Larix* and *Abies*) about a century ago[1] and the early investigation of its structure must have been extremely difficult. In fact, the elucidation of its constitution by Tiemann[2–4] may well be regarded as one of the classical structural investigations. An unusual difficulty was its behaviour on acid hydrolysis, and reference may be made to the cleavage of coniferin to coniferyl alcohol and glucose as one of the first uses of an enzyme in an organic structural investigation.

Coniferin (I) and syringin (II) represent the only two known naturally occurring phenolic

[1] T. HARTIG, *Jahrb. Forst.* **1**, 263 (1861); W. KUBEL, *J. Prakt. Chem.* **97**, 243 (1866).
[2] F. TIEMANN and W. HAARMANN, *Berichte* **7**, 608 (1874).
[3] F. TIEMANN, *Berichte* **8**, 1127 (1875).
[4] F. TIEMANN and N. NAGAI, *Berichte* **8**, 1140 (1875).

glycosides[5–7] derived from *p*-hydroxycinnamyl alcohol, but in view of their function in relation to lignin biosynthesis,[8–10] their fairly wide distribution among plants[11] is not unexpected. Although the distribution of coniferin and syringin is unlikely to be of chemotaxonomic value,[12] it is possible that other glycosides of hydroxycinnamyl alcohols may be isolated and the spectroscopic investigation of the structure of coniferin now reported could be applicable to similar structural problems.

The identification of the compound, m.p. 187°, as coniferin (I) follows from the following evidence. Its i.r. spectrum* suggested that it was a glycoside in that it showed broad absorption due to bonded hydroxyls at 3250 cm^{-1} as well as a sharp band due to a free hydroxyl group at 3500 cm^{-1}. The i.r. spectrum showed no absorption in the carbonyl region, but bands at 1580, 1500, 990, 970, and 860 cm^{-1} indicated the presence of aromatic or unsaturated groupings. Bands at 1250 and 1225 cm^{-1} were attributed to C—O stretching frequencies associated with the glycoside residue. The presence of an aromatic system was clearly supported by its u.v. spectrum† [λ_{max} (in H_2O) 258 nm (ϵ 15,900), 290 nm (4900)] which was compatible with the presence of an oxygenated benzene ring. The pronounced absorption at 258 nm indicated styrenoid conjugation as in isoeugenol [λ_{max} 262 nm (ϵ 13,000), 310 nm (ϵ 3000)].

The mass spectrum of coniferin showed a parent peak at *m/e* 342 and the molecular formula $C_{16}H_{22}O_8$ was selected on the basis of the proton count provided by the NMR spectrum of its pentacetate (see below). The mass spectrum showed a cleavage with hydrogen transfers to give two complementary ions at *m/e* 180 ($C_{10}H_{12}O_3^+$) and *m/e* 162 ($C_6H_{10}O_5^+$). Although the published information on the mass spectrometric behaviour of phenolic glycosides is limited, this cleavage is to be expected[15,16] and suggested that the compound should be represented by the partial structure $C_6H_{11}O_5$—O—$C_{10}H_{11}O_3$.

Acetylation of the compound, m.p. 187°, gave a product, m.p. 115°, and its NMR spectrum showed that it was clearly a pentacetate.‡ It showed three singlets (τ 7·96, 7·92, and 7·90) corresponding with five acetoxyl groups and a singlet (τ 6·18) to be associated with one methoxyl group. The three low field protons (τ 2·91, 3·04, and 3·13) were assumed to be aromatic and they constituted an *ABX* system ($\tau_A = 3{\cdot}13$, quartet, $J_{AX} = 8$ c/sec, $J_{AB} = 2$ c/sec; $\tau_B = 3{\cdot}04$, doublet, $J_{AB} = 2$ c/sec; $\tau_X = 2{\cdot}91$, doublet, $J_{AX} = 8$ c/sec). This required these three aromatic protons to be in the 1,2,4-relationship.

* Some features of the i.r. spectrum of coniferin have been discussed by Hergert.[13]

† The u.v. spectrum of coniferin has been recorded using old nomenclature.[14]

‡ It may be noted that acetylation of coniferin under similar conditions is said to yield a tetracetate, m.p. 125–126°.[4]

[5] R. T. MCILROY, *The Plant Glycosides*, p. 15, Edward Arnold, London (1951).

[6] G. DE STEVENS and F. F. NORD, Natural phenylpropane derivatives, in *Modern Methods of Plant Analysis* (edited by K. PAECH and M. V. TRACEY), p. 396, Springer-Verlag, Berlin (1955).

[7] W. KARRER, *Konstitution und Vorkommen der organischen Pflanzenstoffe*, pp. 108 and 110, Birkhäuser Verlag, Basel (1958).

[8] S. A. BROWN, Lignin and tannin biosynthesis, in *Biochemistry in Phenolic Compounds* (edited by J. B. HARBORNE), p. 372, Academic Press, London and New York (1964).

[9] K. FREUDENBERG, *Nature* **183**, 1152 (1959).

[10] T. SWAIN, in *Wood Extractives* (edited by W. E. HILLIS), p. 277, Academic Press, New York (1962).

[11] V. PLOUVIER, *Compt. Rend.* **236**, 1577 (1952); **238**, 1835 (1954); **254**, 4196 (1962).

[12] R. PARIS, The distribution of plant glycosides, in *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 343, Academic Press, London and New York (1963).

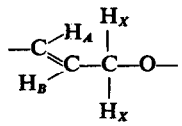
[13] H. L. HERGERT, *J. Org. Chem.* **25**, 405 (1960).

[14] R. F. PATTERSON and H. HIBBERT, *J. Am. Chem. Soc.* **65**, 1862 (1943).

[15] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structural Elucidation by Mass Spectrometry*, Vol. 2, p. 224, Holden-Day, San Francisco (1964).

[16] I. A. PEARL and S. F. DARLING, *Tetrahedron Letters* 1869 (1967).

Four protons [$\tau_A = 3{\cdot}37$ (doublet); $\tau_B = 3{\cdot}85$ (multiplet), and $\tau_{X_2} = 5{\cdot}29$ (doublet)] formed an ABX_2 system with $J_{AB} = 16$ c/sec and $J_{BX} = 5$ c/sec. The chemical shifts and multiplicities were compatible with the presence of the grouping



in which the double bond is conjugated with the aromatic ring. Incidentally, it may be noted that the coupling constant $J_{AB} = 16$ c/sec requires the double bond to be *trans* in coniferin. Integration established the presence of seven protons associated with the glycosidyl residue but detailed assignments could not be made.

Thus on this evidence the compound, m.p. 187°, could be regarded as either 3-methoxy-4-glycosidyloxycinnamyl alcohol or 4-methoxy-3-glycosidyloxycinnamyl alcohol. The nature of the sugar residue and the identity of the compound as coniferin was established by its enzymatic cleavage which yielded glucose and coniferyl alcohol (III). The coniferyl alcohol was characterized as its monomethyl ether and its NMR spectrum is appropriately related to the NMR spectrum of coniferin pentacetate. As expected, the doublet due to the allylic methylene group is at higher field in the alcohol (τ 5·87) than in the acetate (τ 5·29).

These results establish the structure and stereochemistry of the compound, m.p. 187°, isolated from *B. polyandra* Griff. as shown in formula I and establish its identity as coniferin.

EXPERIMENTAL

Isolation of Coniferin (I) from Balanophora polyandra *Griff.*

The plant material was collected in Thailand and the total aerial parts were dried, ground (1 kg), and continuously extracted (48 hr) with hot light petroleum (b.p. 40–60°), to remove waxy materials. Further continuous extraction (48 hr) with hot methanol gave an extract which was concentrated and then diluted with water. After keeping overnight (0–5°), the crystalline precipitate was collected and further crystallization from water gave coniferin (10·2 g) as the dihydrate, m.p. 187° (Found: C, 50·75; H, 6·90. Calc. for $C_{16}H_{22}O_8.2H_2O$: C, 50·79; H, 6·93 per cent). $[\alpha]_D^{20}$(MeOH) = −60°.

Coniferin Pentacetate

Coniferin (200 mg) was dissolved in pyridine (1 ml) and acetic anhydride (2 ml) added. After standing at room temperature (12 hr) the mixture was poured on to ice, lyophilized, and the residue crystallized from methanol. This gave *coniferin pentacetate* (180 mg) as colourless prisms, m.p. 115° (Found: C, 56·68; H, 5·94. $C_{26}H_{32}O_{18}$ required: C, 56·72; H, 5·82 per cent).

Hydrolysis of Coniferin

β-Glucosidase (5 mg; L. Light and Co. Ltd.) was added to an aqueous sodium acetate–acetic acid buffer solution (pH = 6) (25 ml) containing coniferin (250 mg) and the temperature of the mixture was maintained at 36°. During 48 hr the course of the reaction was followed by TLC and at the end of this period no coniferin could be detected. The mixture was then extracted with $CHCl_3$ and gave coniferyl alcohol (III) (83 mg), m.p. 73° (lit. m.p. 74°[3]), λ_{max} (in H_2O) 262 nm (ε 15,400), 294 nm (ε 5500). The coniferyl alcohol was characterized by reaction with CH_2N_2 in methanol to give 3,4-dimethoxycinnamyl alcohol. Its NMR spectrum showed the following features: broad singlet τ 6·98 (OH); singlet τ 6·2 $(OMe)_2$; doublet τ 5·87, $J = 5$ c/sec (CH_2); multiplet τ 3·85 (Ar—CH=CH); doublet τ 3·45, $J = 16$ c/sec (Ar—CH=CH—); *ABX* system ($\tau_A = 3{\cdot}03$, $\tau_B = 3{\cdot}05$, $\tau_X = 3{\cdot}20$; $J_{AX} = 8$ c/sec, $J_{AB} = 2$ c/sec, $J_{BX} \sim 0$ c/sec) (three aromatic protons).

The coniferyl alcohol monomethyl ether gave a parent peak at *m/e* 194 ($C_{11}H_{14}O_3$ requires M, 194).

The aqueous solution was then examined by two-dimensional paper chromatography using (1) 6 per cent acetic acid and (2) *sec*-butanol–acetic acid–water (14:1:5) as developing solvent. Spraying with aniline hydrogen phthalate solution revealed one carbohydrate component whose chromatographic behaviour was identical with that of glucose.