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AN ISOFLAVONE AND A COUMESTAN FROM *EYSENHARDTIA POLYSTACHYA*—ROBERT BOYLE'S FLUORESCENT ACID–BASE INDICATOR

DUNCAN T BURNS, BARRY G DALGARNO, PAUL E GARGAN and JAMES GRIMSHAW

Department of Chemistry, Queen's University, Belfast BT9 5AG, Northern Ireland, U K

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Abstract—7-Hydroxy-2',4',5'-trimethoxyisoflavone is the principal fluorescent phenolic constituent of the heartwood of *Eysenhardtia polystachya*, Robert Boyle's fluorescent acid–base indicator The bark yielded 9-methoxy-2,3-methylenedioxycoumestan

INTRODUCTION

Eysenhardtia polystachya (Ortego) Sarg (Leguminosae Galegeae) is a small tree distributed in Mexico and Texas which has been used since pre-Colombian times in the treatment of kidney and bladder infections and as a diuretic [1] The manuscript history of this material from Colombian times was reviewed in detail by Partington [2] The wood was exported to Europe as *lignum nephriticum* in the sixteenth and seventeenth centuries and attracted attention on account of the apparent medical virtues and for the unusual yellow colour and pronounced blue fluorescence displayed by its infusion in water Aqueous extract of the wood are of historical interest, being the fluorescent acid–base indicator used by Robert Boyle [3, 4] in the seventeenth century (although the term fluorescence was not introduced until much later) By the mid-eighteenth century the wood had become rare in Europe and its botanical origin was lost Safford [5] positively identified the material used by Boyle The structure of the principal fluorescent component of the heartwood is elucidated here A previous extraction of the dried stems and bark by Mexican workers [6] yielded 3,4-dimethoxy-8,9-methylenedioxypterocarpan (**1**), dehydrorotenone (**2**) and a fluorescent glycoside, angustlegorretoside, of unknown structure

RESULTS AND DISCUSSION

A methanol extract of heartwood showed eight components on TLC (silica gel) Three components fluoresced under UV and were extracted into aqueous sodium hydroxide and precipitated after acidification After purification by TLC (silica gel), the most abundant fluorescent component was obtained as a solid, mp 234–237°

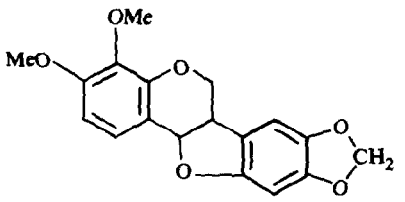
The mass spectrum indicated a molecular formula of $C_{18}H_{18}O_6$ ($[M]^+$ at 328 and loss of Me and CO) and the ^1H NMR spectrum showed the presence of three methoxy groups, which together with a phenolic group accounted for four of the six oxygen atoms IR carbonyl absorption at 1621 cm^{-1} and UV λ_{max} at 244 and 295 nm are characteristic [7] of the isoflavone skeleton and the UV absorption was moved to longer wavelengths on addition of sodium hydroxide The ^1H NMR spectrum showed three singlets, at δ 7 94 due to the hydrogen at C-2 of the isoflavone ring, and at δ 6 62 and 6 94 due to the remaining aromatic protons on a 1,2,4,5-tetrasubstituted benzene ring The low-field doublet at δ 8 19 can be assigned to the proton at C-5, deshielded by the adjacent carbonyl group The *ortho*-coupling constant then identifies the proton at C-6 which in turn is *meta*-coupled to a proton at C-8 The pattern of oxygen substitution in this isoflavone is therefore unambiguous as in structure **3** A comparison with the NMR spectra of other flavones and isoflavones enabled assignment of the hydroxy- and methoxy-substituents as given in **3** with a 7-hydroxy-substituent A 7-methoxy-substituent would cause the proton at C-8 to appear with δ 6 98

At pH 8 04, a solution of **3** in aqueous methanol showed a strong blue fluorescence with λ_{max} 480 nm This fluorescence was reduced to half the intensity at pH 6 8 and was replaced at pH 5 3 by a weaker fluorescence with λ_{max} 440 nm, almost invisible to the eye A crude extract of the heartwood showed similar behaviour Isoflavone shows a weak fluorescence at 77K with λ_{max} 485 nm [8]

Isoflavone **3** is known from synthesis [9] with mp 244–245° and it has been shown [10] to be a precursor for amorphigenin in *Amorpha fruticosa* L (Leguminosae Galegeae) seedlings It was not isolated from this source but trace amounts were detected by an isotopic dilution technique *Amorpha* and *Eysenhardtia* species are closely related botanically

Extraction of the bark from mature logs of *E polystacha* (only 200 g was available) with petrol and then methanol yielded only one fluorescent component, mostly present in the petrol extract and isolated by TLC (silica gel) as needles subliming at 205° No angustlegorretoside was found

The material showed IR absorption at 1740 cm^{-1} due to a carbonyl group and UV λ_{max} 244, 309 and 347 nm unchanged after addition of sodium hydroxide indicating the absence of a phenolic group The mass spectrum indicated the molecular formula $C_{17}H_{10}O_6$ ($[M]^+$ with fragmentation due to loss of Me and CO) The ^1H NMR spectrum indicated one methylenedioxy and one methoxy group, two aromatic proton singlets due to the remaining



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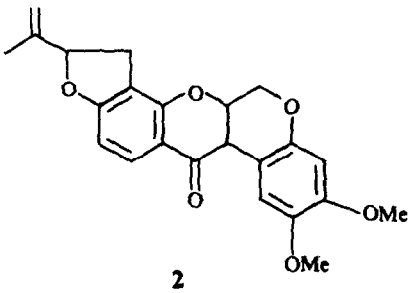

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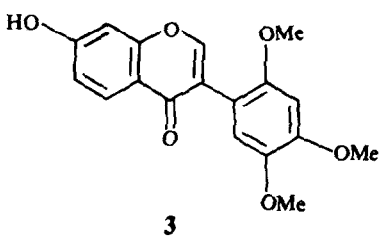

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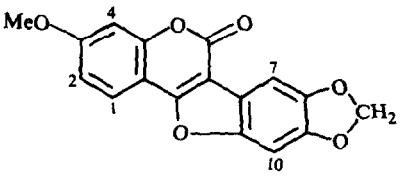

4

protons on a 1,2,4,5-tetrasubstituted benzene ring and three other coupled resonances due to a 1,2,4-arrangement of protons on an aromatic ring The molecular fragment so far unaccounted for corresponded to the coumestan skeleton and the IR and UV data were consistent with such a chromophore [11–14] Thus the material must have either structure 4 or the reverse substitution pattern of 9-methoxy-2,3-methylenedioxycoumestan The proton singlets in the NMR spectrum (δ 7 35 and 7 37) were at a similar position to singlets due to protons at C-7 and C-10 in the spectrum of 3,8,9-trimethoxycoumestan (δ 7 35 and 7 56) [14] and quite distinct from singlets due to protons at C-1 and C-4 in the spectrum of 2,3,8-trimethoxycoumestan (δ 7 11 and 6.96) [15] The coumestan from *E polystacha* bark was therefore assigned structure 4 As flemichapparin-C, mp 272°, it has been isolated from *Flemingia chappar* Buch -Ham (Leguminosae Lotoideae) [16] and a synthesis is reported [17] Published IR and UV data agree with the data here, solubility problems prevented the Indian workers from obtaining NMR spectral data Compound 4 showed a bluish fluorescence in aqueous methanol with λ_{max} 445 unchanged over the pH range 9.2–6 5 and at the same wavelength but with diminished intensity at pH 4.0 It would make no contribution to Boyle's fluorescent indicator

In the middle of the seventeenth century a second *lignum nephriticum* was imported to Europe from the Philippines and for part of its journey was transported overland through Mexico The two woods both give fluorescent infusions and became confused with each other Their history is disentangled by Safford, the second wood, *Pterocarpus indicus* Willd (Leguminosae Dalbergieae), has been examined chemically and 7-hydroxy-4'-methoxyisoflavone isolated from the heartwood [18] The workers do not comment on the fluorescence of this isoflavone which must be expected to be similar to that shown by 3 thus accounting for the closely similar appearance of infusions of the two woods

EXPERIMENTAL

An authentic supply of *E polystachya* in the form of logs 6–8 cm diameter was obtained from the Instituto Nacional de Investigaciones sombre Recursos Bioticos, Xalapa (Vera Cruz province) by courtesy of H M Ambassador to Mexico In a coincidence with the events of 1519, Cortes passed through Xalapa during his march from the coast to Mexico City [19] Aq methanolic extracts of the dense brown heartwood showed a blue fluorescence which was not observed from infusions of the white sap wood

Powdered heartwood (500 g) was Soxhlet-extracted with MeOH for 96 hr and the extract concd Portions of extract (20 g) were dissolved in petrol (200 ml) and extracted with 5 M NaOH The alkaline extract was acidified and the precipitated material (1 4 g) isolated with Et_2O TLC on silica gel and elution with EtOAc–C_6H_6 (3 2) showed three fluorescent components, R_f 0 36, 0 30 and 0 25 Two prep TLC (silica gel) stages afforded the most abundant component, R_f 0 30 Crystallization from $CHCl_3$ gave 7-hydroxy-2',4',5'-trihydroxyisoflavone, mp 234–237°, UV λ_{max}^{MeOH} nm (ε) 244 (7500), 297 (5700), IR ν_{max}^{KBr} cm^{-1} 1621, MS *m/z* (rel int) 328 09464 (100) ($C_{18}H_{16}O_6$), 313 (20), 297 (21), 285 (11), 255 (13), 253 (16), 148 (14), 105 (36), ^{1}H NMR ($CDCl_3$) δ 8 19 (1H, *d*, J = 6 9 Hz, H-5), 7 94 (1H, *s*, H-2), 6 94 (1H, *s*, H-2'), 6 90 (1H, *q*, J = 6 9 and 3 0 Hz, H-6), 6 86 (1H, *d*, J = 3 0 Hz, H-8), 6 02 (1H, *s*, H-5'), 3 93 (3H, *s*, OMe), 3 86 (3H, *s*, OMe), 3 78 (3H, *s*, OMe).

7-Hydroxy-4'-methoxyisoflavone [20] had ^{1}H NMR δ 8 02 (H-5), 6 97 (H-6), 6 88 (H-8), 2',4',5',6,7-pentamethoxy isoflavone [21] had ^{1}H NMR 6 87 (H-6'), 6 63 (H-3')

Powdered bark (200 g) was extracted with petrol (1 l) for 100 hr and then with MeOH (1 l) for 100 hr Both extracts were examined by TLC on cellulose plates eluted with MeOH and silica gel plates eluted with C_6H_6 saturated with H_2O Only one fluorescent material was present, R_f 0 31 on cellulose and 0 26 on silica gel, it was more abundant in the petrol extract Evapn and prep TLC on silica gel afforded the fluorescent material as needles (3 mg) sublimed at 205°/2 mm Hg UV λ_{max}^{MeOH} nm (ε) 244 (16 000), 309 (9000), 347 (22 000), IR ν_{max}^{KBr} cm^{-1} 1740, MS *m/z*

(rel int) 310 04825 (100), $C_{17}H_{10}O_6$, 295 (33), 267 (20), ^{1}H NMR ($CDCl_3$) δ 7 95 (1H, *d*, *J* = 9 3 Hz, H-5), 7 37 (1H, *s*, H-10), 7 35 (1H, *s*, H-13), 7 10 (1H, *d*, *J* = 2 2 Hz, H-8), 7 10 (1H, *q*, *J* = 9 3 and 2 2 Hz, H-6), 6 16 (2H, *s*, CH_2O_2), 3 98 (3H, *s*, OMe)

REFERENCES

1 Martinez, M (1959) *Plantas Medicinales de Mexico*, 5th edn, p 469 Editorial Botas, New York
2 Partington, J R (1955) *Ann Sci* **11**, 1
3 Boyle, R (1664) *Experiments and Considerations Touching Colours* H Herringman, Anchor, New Exchange, London
4 Boyle, R (1684/5) *Short Memoirs for the Natural Experimental History of Mineral Waters* S Smith, London
5 Safford, W E (1915) *Annu Rep Smithsonian Inst* 271
6 Dominguez, X A, Franco, R and Diaz Viveros, Y (1978) *Rev Latinoam Quim* **9**, 209
7 Mabry, T J (1969) in *Perspectives in Phytochemistry* (Harborne, J B and Swain, T, eds) p 22 Academic Press, London
8 Efimov, A A and Nurmukametov, R N (1971) *Opt Spektrosk* **30**, 622
9 Fukui, K, Nakayama, M, Hatanaka, M, Okamoto, T and Kawase, Y (1963) *Bull Chem Soc Jpn* **36**, 397
10 Crombie, L, Dewick, P M and Whiting, D A (1973) *J Chem Soc Perkin Trans* 1, 1285
11 Livingston, A L, Witt, S C, Lundin, R F and Bickoff, E M (1965) *J Org Chem* **30**, 2353
12 Spencer, R R, Bickoff, E M, Lundin, R E and Knuckles, B E (1966) *J Agric Food Chem* **14**, 162
13 Zilg, H and Grisebach, H (1968) *Phytochemistry* **17**, 1765
14 Kurosawa, K and Nogami, K (1976) *Bull Chem Soc Jpn* **49**, 1955
15 Simonova, L (1972) *Khim Prir Soedin* **8**, 436
16 Adityachaudhury, N and Gupta, P K (1973) *Phytochemistry* **12**, 426
17 Farkas, L, Antus, S and Nogradi, M (1974) *Acta Chim Hung* **82**, 225
18 Cooke, R G and Rae, I D (1964) *Aust J Chem.* **17**, 379
19 de Madariaga, S (1955) *Hernan Cortes, Conqueror of Mexico* Henry Regnery, Chicago
20 Batterham, T J and Highet, R J (1964) *Aust J Chem* **17**, 428
21 Campbell, R V M, Harper, S H and Kemp, A D (1969) *J Chem Soc C* 1787