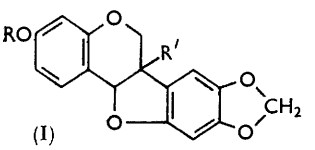
957. *Extractives from Woods. Part I. Extractives from* Andira inermis (*Wright*) *H.B.K.**

By Wesley Cocker, Thor Dahl, (Miss) Carmel Dempsey, and T. B. H. McMurry.

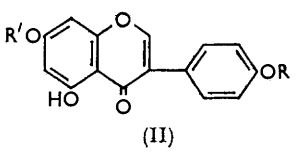
A new isoflavonoid (I; R = R′ = H), related to pterocarpin (I; R = Me, R′ = H), has been isolated from *Andira inermis* (Wright) H.B.K. Biochanin A (II; R = Me, R′ = H) is also present in this timber, together with small quantities of long-chain, unbranched fatty acids.

Some two years ago we started an investigation of the extractives from woods found in the British West Indies. *Andira inermis* (Wright) H.B.K. is known in Trinidad as angelin, and in British Guiana as koraro (bat seed), and it belongs to the family Leguminosae (Papilionaceae). Other species, namely, *A. araroba*,[1] *A. excelsa* = *Vouacapoua americana*,[2] *A. retusa* = *Geoffroya surinamensis*,[3] and *A. spectabilis* = *Ferreirea spectabilis*,[3,4] have been investigated by others. *A. inermis* is found in marshy woodland. The wood is a powerful anthelmintic, a narcotic, purgative, and poisonous.[5]

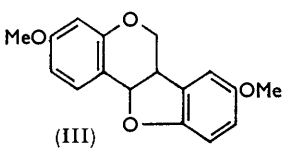
The family Leguminosae (Papilionaceae) is a rich source of isoflavonoids (I) and (II) and related structures. Thus pterocarpin[6] (I; R = Me, R′ = H) has been isolated from *Pterocarpus santalinus* L.,[7] *P. dabergoides*, *P. macrocarpus*,[8] and *Baphia nitida*,[9] and homopterocarpin (III) from *P. santalinus*[7] and *P. soyauxii*.[8] Pisatin, to which



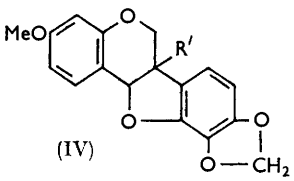
(I)



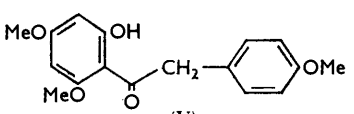
(II)



(III)



(IV)



(V)

structure (IV; R′ = OH) was given[10] on the basis of the earlier structure (IV; R′ = H) of pterocarpin and should undoubtedly be represented by (I; R = Me, R′ = OH), has been isolated from *Pisum sativum* L. Biochanin A (II; R = Me, R′ = H) is obtained from *A. spectabilis*,[4] *Trifolium pratense*, and *T. subterraneum*,[11] and prunetin (II; R = H, R′ = Me) and genistein (II; R = R′ = H) from *Pterocarpus angolensis*[12] and various species of *Trifolium*.[11,13]

* Preliminary publication: *Chem. and Ind.*, 1962, 216.

[1] Karrer, "Konstitution und Vorkommen der organischen Pflanzenstoffe," Birkhaüser Verlag, Basle, 1958, p. 505.
[2] Ref. 1, p. 794; King, Godson, and King, *J.*, 1955, 1117.
[3] Ref. 1, p. 964.
[4] King, Grundon, and Neill, *J.*, 1952, 4580.
[5] Information kindly supplied by the Tropical Products Institute of the D.S.I.R.
[6] Bredenberg and Shoolery, *Tetrahedron Letters*, 1961, No. 9, 285.
[7] Cazeneuve, *Ber.*, 1874, **7**, 1798.
[8] King, Cotterill, Godson, Jurd, and King, *J.*, 1953, 3693.
[9] McGookin, Robertson, and Whalley, *J.*, 1940, 787.
[10] Cruickshank and Perrin, *Nature*, 1960, **187**, 799; Perrin and Bottomley, *ibid.*, 1961, **191**, 76.
[11] Pope and Wright, *Chem. and Ind.*, 1954, 1019.
[12] King, King, and Warwick, *J.*, 1952, 1920; King and Jurd, *J.*, 1952, 3211.
[13] Curnow, *Biochem. J.*, 1954, **58**, 283; Curnow and Rossiter, *Australian J. Exp. Biol. Med. Sci.*, 1955, **33**, 243.

Extraction of *A. inermis* wood with ligroin gave a mixture of phenols, one of which consisted of the strongly lævorotatory isoflavonoid (I; R = R′ = H) which we name inermin. We propose, however, to replace this name by demethylpterocarpin. The compound crystallises from aqueous ethanol as a hemihydrate, $C_{16}H_{12}O_5,\frac{1}{2}H_2O$, and as the unhydrated compound from benzene. It readily gives a monomethyl ether, identical with pterocarpin (I; R = Me, R′ = H).

There was a wide variation in the demethylpterocarpin content of the two batches of *A. inermis* used: 0·2% in a batch from Trinidad, 0·047% in one from British Guiana.

Since this work was completed Bredenberg and Hietala [14] isolated the glycoside, trifolirhizin (I; R = glucose, R′ = H) from *Trifolium pratense* and hydrolysed it enzymically to the aglycone which is undoubtedly demethylpterocarpin. More recently Suginome [15] described the isolation and synthesis of maackiain, from *Maackia amunensis*, which is identical with " inermin " (it is this dual nomenclature which induced us to change the name to demethylpterocarpin).

Biochanin A (II; R = Me, R′ = H), isolated in 0·06% yield from the phenolic fraction of the ligroin extract of *A. inermis* (Trinidad), was identified by its spectrum, by alkaline hydrolysis of its dimethyl ether to the known deoxybenzoin (V),[16] and by synthesis.[17] An unidentified mixture of phenols, which could not be resolved, was also isolated; its specific rotation and ultraviolet spectrum were characteristic of demethylpterocarpin, but a maximum at 2080 and a shoulder at 2280 Å which it displayed could be derived from the presence of biochanin A in the mixture.

Small quantities of unbranched fatty acids from C_{20} to C_{26} inclusive, and C_{29} and C_{30} are present in the ligroin extract of *A. inermis*. They were identified by gas chromatography of their methyl esters. All the odd-numbered saturated acids from C_{22} to C_{32} have previously been found [18] in saponified montan wax and the C_{21} and C_{23} acids are present in butter fat,[19] though other sources [20] have stated that C_{23}, C_{25}, and C_{29} acids are not found in Nature. Whilst arachidic (eicosanoic) acid constitutes over 70% of the acid mixture from *A. inermis* very significant quantities of heneicosanoic acid are also present.

Experimental

Pulverised heartwood (3 kg.) of *Andira inermis*, from Trinidad, was extracted continuously for 48 hr. with boiling ligroin (b. p. 60—80°) (2·5 l.). The extract was concentrated to 200 c.c. and set aside overnight at 0°, and the deposited solid was collected and washed with ice-cold ligroin. The solid was dissolved in ether which was extracted with 5% aqueous sodium hydrogen carbonate (4 × 50 c.c.) and then with 5% aqueous sodium hydroxide (4 × 50 c.c.). The former extract gave on acidification a black tar (0·5 g.) which was not further investigated. The latter extract was acidified and extracted with ether, from which a solid was obtained. This crystallised from dilute alcohol, giving *demethylpterocarpin hemihydrate* as plates (6 g.), m. p. 100—105° and 174—175°, $[\alpha]_D^{20}$ −221° (*c* 0·22 in MeOH), $\lambda_{max.}$ 2860 (log ε 3·72) and 3090 Å (log ε 3·93), $\nu_{max.}$ 3500 (OH), 1626, 1592, 1504 (aromatic), 847, and 709 cm.$^{-1}$ (CH_2O_2:) (Found: C, 65·9, 65·3; H, 4·6, 4·5. $C_{16}H_{12}O_5,\frac{1}{2}H_2O$ requires C, 65·5; H, 4·5%). Further crystallisation from benzene afforded the anhydrous *compound* as needles, m. p. 179—180° (Found: C, 67·8; H, 4·3; OMe, 0. $C_{16}H_{12}O_5$ requires C, 67·6; H, 4·3%).

Demethylpterocarpin (100 mg.) was heated at 100° for 90 min. with acetic anhydride (1 c.c.) and pyridine (1 c.c.); the *acetate* (100 mg.) was obtained as needles (from ethyl acetate), m. p. 178° (depressed by demethylpterocarpin), $[\alpha]_D^{19}$ −179° (*c* 0·166 in $CHCl_3$), $\lambda_{max.}$ 2830 and 3090 Å

[14] Bredenberg and Hietala, *Acta Chem. Scand.*, 1961, **15**, 696, 936.
[15] Suginome, *Experientia*, 1962, **18**, 161.
[16] Robertson, Suckling, and Whalley, *J.*, 1949, 1571.
[17] Baker, Chadderton, Harborne, and Ollis, *J.*, 1953, 1852.
[18] Hewett, Kipping, and Jeffery, *Nature*, 1961, **192**, 65.
[19] Hansen, Shorland, and Cooke, *J. Dairy Res.*, 1959, **26**, 190.
[20] Ralston, " Fatty Acids and their Derivatives," Wiley and Sons, Inc., New York, 1948, pp. 48, 51; Markley, " Fatty Acids," Interscience Publ., Inc., New York, 1961, p. 32.

(log ε 3·47 and 3·78, respectively), $\nu_{max.}$ 1755 (ester), 1615, 1591, 1495, 1228 (OAc), 847, and 711 cm.$^{-1}$ (Found: C, 65·8; H, 4·4. $C_{18}H_{14}O_6$ requires C, 66·2; H, 4·3%).

Demethylpterocarpin (200 mg.) in methanol (10 c.c.) was set aside with diazomethane in ether. The methyl ether (200 mg.) crystallised from ethyl acetate–light petroleum as rhombs, m. p. 159—160° alone or mixed with pterocarpin, $[\alpha]_D^{17}$ −221° (*c* 0·47 in $CHCl_3$), $\lambda_{max.}$ 2850 and 3100 Å (log ε 3·44 and 3·69, respectively), $\nu_{max.}$ 1626, 1587, 1504, 1163, 1143, 1031, 926, 847, and 711 cm.$^{-1}$ [authentic pterocarpin had $\lambda_{max.}$ 2870 and 3120 Å (log ε 3·30 and 3·40, respectively) and an infrared spectrum identical with that of " *O*-methylinermin "] (Found: C, 68·2; H, 4·9; OMe, 10·0. Calc. for $C_{17}H_{14}O_5$: C, 68·45; H, 4·7; OMe, 10·4%).

Biochanin A.—Evaporation of the ethanolic mother-liquors from which demethylpterocarpin hydrate had been deposited afforded a gum (4 g.). This was chromatographed in light petroleum (b. p. 40—60°; 60 c.c.) on silica gel (15 × 3 cm.), and eluted with (*a*) benzene and (*b*) benzene–ether (9 : 1). The former gave a gum which became an off-white solid (1·7 g.), m. p. 112—113°, $[\alpha]_D^{20}$ −229·5° when ground with ligroin but could not be satisfactorily crystallised. It showed maxima at 2080, 2880, and 3100 Å, and gave a weak methylenedioxy-reaction (Found: C, 69·3; H, 5·25; OMe, 8·8%). Reaction with acetic anhydride (5 c.c.) and pyridine (20 c.c.) for 2 hr. at 100° gave an oil which became solid (1·7 g.) on treatment with ether and had m. p. 128—130°. Two crystallisations from ethanol gave needles (0·75 g.), m. p. 115—116° raised to 148—150°, $[\alpha]_D$ −197·3°, $\lambda_{max.}$ 2080, 2280 infl., 2880, and 3100 Å. (Found: C, 68·4, 68·2, 67·7; H, 4·5, 4·9, 5·2; OMe, 5·1; Ac, 21·4%). Eluant (*b*) gave a solid (0·5 g.), m. p. 209—210° which crystallised from ethanol as yellow needles, m. p. 210°,[21] undepressed by an authentic specimen of biochanin A, $\lambda_{max.}$ (in EtOH) 2620 (log ε 4·52) and 3300 Å (log ε 3·58), $\lambda_{max.}$ (in EtOH–$AlCl_3$) 2100 (log ε 4·36), 2300, infl. (log ε 4·09), 2740 (log ε 4·53), 3100, infl. (log ε 3·78) and 3800 Å (log ε 3·58), $\lambda_{max.}$ (in EtOH–NaOAc–H_3BO_3) 2180 (log ε 4·31), 2460, infl. (log ε 4·15), 2760 (log ε 4·56), and 3360 Å (log ε 3·98), $\nu_{max.}$ (in Nujol) 3500 and 3400 (OH), 1580, 1525, and 770 cm.$^{-1}$ (Found: C, 67·5; H, 4·3; OMe, 11·4, 11·1. Calc. for $C_{16}H_{12}O_5$: C, 67·6; H, 4·3; 1OMe, 10·9%). Its dimethyl ether, prepared by refluxing the phenol (123 gm.) in dry acetone with fused potassium carbonate (5 g.) and dimethyl sulphate (1 c.c.) for 18 hr., crystallised from ethyl acetate as diamonds, m. p. 161°,[21] $\lambda_{max.}$ 2090 (log ε 4·28), 2150, infl. (log ε 3·68), and 2580 Å (log ε 4·46) (Found: C, 69·4; H, 5·5; OMe, 29·8, 30·0. Calc. for $C_{18}H_{16}O_5$: C, 69·2; H, 5·2; 3OMe, 29·8%). Its acetate crystallised from ethyl acetate as colourless needles, m. p. 191·5—192·5°[21] (Found: C, 65·1; H, 4·5; OMe, 8·3. Calc. for $C_{20}H_{16}O_7$: C, 65·2; H, 4·4; 1OMe, 8·3%).

2-Hydroxy-4,6,4′-trimethoxydeoxybenzoin.—Biochanin A (68·7 mg.) was refluxed, under nitrogen, in ethanol (24 c.c.) with potassium hydroxide (70 mg.) for 50 min. Removal of solvent and extraction of the residue with ether gave an oil. This was dissolved in a hot mixture of ligroin and ethyl acetate and then cooled, giving the deoxybenzoin, m. p. and mixed m. p. 86°,[16] $\lambda_{max.}$ 2220 (log ε 4·3) and 2900 Å (log ε 4·3) (Found: C, 67·7; H, 5·3; OMe, 30·3. Calc. for $C_{17}H_{18}O_5$: C, 67·5; H, 6·0; 3OMe, 30·8%).

Fatty Acids.—*A. inermis* (54 lb.) from British Guiana was extracted with ligroin as described above. The extract was washed with 5% aqueous sodium hydroxide from which a dark brown oil (3·65 g.) was obtained by acidification and extraction with ether. The oil was chromatographed on a silica gel column and eluted with 4 : 1 benzene–light petroleum, giving a solid (480 mg.) which crystallised from methanol in plates, m. p. 72—74°, $\nu_{max.}$ 3077 (bonded OH) and 1709 cm.$^{-1}$ (CO_2H), 727, 719 (aliphatic chain). This compound (80 mg.) was esterified in ether with fresh diazomethane, giving a solid which crystallised from methanol in plates, m. p. 83—86°, $[\alpha]_D$ 0°. This was chromatographed by using an Aerograph, Hy Fi 600 (Wilkens Instrument and Research Inc.), under the conditions given below. Synthetic methyl esters were used as standards.

Vanillin was isolated (2 mg.) as its 2,4-dinitrophenylhydrazone, m. p. 269—271°, R_F 0·052, by thin-layer chromatography on silica gel (ether as solvent system), from the ligroin extract of *A. inermis* (British Guiana) after extraction with 5% sodium carbonate solution.

β-Sitosterol (47 mg.), m. p. and mixed m. p. 136—138°, $\nu_{max.}$ 3509 (OH), 1653 cm.$^{-1}$ (C=C), was also isolated from the neutral fraction of this wood by elution from silica gel with 9 : 1 ether–acetone.

[21] Bose and Siddiqui, *J. Sci. Ind. Res., India*, 1945, **4**, 231; cf. King, Grundon, and Neill, *J.*, 1952 4580.

Composition of mixture of methyl esters.

Conditions of chromatography: column, Silicone rubber, 5′ × ⅛″; 251°; chart speed, 20 in./hr.; N_2 flow, 20 c.c./min., H_2 flow, 24 c.c./min.

Peak no.	Relative retention time	Retention vol. (ml.)	Fatty acids (as Me esters) (Me behenate = 1)	Approx. quantity (%)
1	0·64	36	Arachidic	72·0
2	0·79	42	Heneicosanoic	15·7
3	1·0	54	Behenic	3·7
4	1·25	66·3	Tricosanoic	2·3
5	1·57	90	Lignoceric	3·6
6	2·07	114	Pentacosanoic	1·5
7	2·68	138	Cerotic	1·0
8	5·63	312	Nonacosanoic	0·2
9	6·53	360	Melissic	0·1

The authors thank Professor W. B. Whalley for a specimen of pterocarpin, The Tropical Products Institute of the Department of Scientific and Industrial Research and The Conservator of Forests, British Guiana, for providing the wood, the U.S. Army through its European Research Office for supporting this investigation, and the Forest Products Research Laboratory, Princes Risborough, for identifying the *Andira inermis*.

UNIVERSITY CHEMICAL LABORATORY, TRINITY COLLEGE,
DUBLIN, REPUBLIC OF IRELAND.

[*Received, May 30th*, 1962.]