Isoflavonoid Constituents of the Heartwood of *Cordyla africana*

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Three further compounds have been isolated from the heartwood of *Cordyla africana* (Leguminosae), and shown by chemical degradation and spectral considerations to be 6-hydroxy-2',7-dimethoxy-4',5'-methylenedioxy-5,6,7-trimethoxy-3',4'-methylenedioxy-, and 5,6,7,8-tetramethoxy-3',4'-methylenedioxy-isoflavone. The mass spectra of these, and related isoflavones are discussed.

PREVIOUS examination of the ether extract of the heartwood of *Cordyla africana* (Leguminosae; sub-family: Caesalpinioideae, tribe: Swartzieae) afforded several new isoflavones and an isoflavanone.[1] We now report the isolation of a further three new isoflavones, from the chloroform extract of the heartwood of *Cordyla africana*.

T.l.c. of the alkali-soluble fraction of the chloroform extract afforded a crystalline phenolic compound (I; R = H) [compound (7)], $C_{18}H_{14}O_7$. The i.r. and u.v. spectra indicated an isoflavonoid structure,[1,2] and the n.m.r. spectrum revealed the presence of a methylenedioxy- and two methoxy-groups. Methylation with diazomethane gave 2',6,7-trimethoxy-4',5'-methylenedioxyisoflavone (I; R = Me), identical with an authentic sample previously isolated from *Cordyla africana*.[1]

The mass spectrum of the compound (Table) exhibits an $M - 31$ peak in high abundance, indicating a methoxy-group in the 2'-position,[1,3,4] and the presence of the

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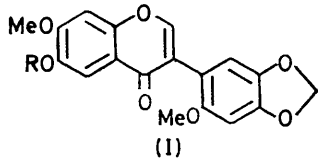
[1] R. V. M. Campbell, S. H. Harper, and A. D. Kemp, *J. Chem. Soc.* (*C*), 1969, 1787.

[2] W. D. Ollis, 'The Chemistry of Flavonoid Compounds,' ed. T. A. Geissman, Pergamon, Oxford, 1962, p. 366; F. M. Dean 'Naturally Occurring Oxygen Ring Compounds,' Butterworth, London, 1963, p. 370.

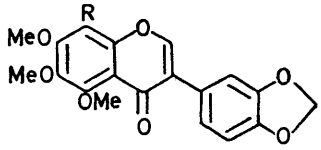
[3] W. D. Ollis, C. A. Rhodes, and I. O. Sutherland, *Tetrahedron*, 1967, **23**, 4741.

[4] R. I. Reed and J. M. Wilson, *J. Chem. Soc.*, 1963, 5949.

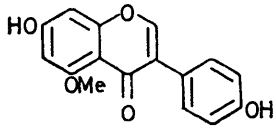
hydroxy-group in ring A is substantiated by peaks at *m/e* 167 and 175 arising from retro-Diels–Alder fission of the heterocyclic ring. The absence of any shift in the



(I)
R=H or Me



(II)
R=H or OMe



(III)

u.v. absorption maxima on treatment with fused sodium acetate indicates [5] the absence of a free hydroxy-group at position 7, thus placing the hydroxy-group at position 6; this was confirmed by fusion of the compound with potassium hydroxide, which gave 4-hydroxy-3-methoxyphenol.

Column chromatography of the alkali-insoluble fraction of the chloroform extract, followed by t.l.c., afforded 2′,4′,5′,6,7-pentamethoxyisoflavone (1), 3′,4′,6,7-tetramethoxyisoflavone (2), 3′,6,7-trimethoxy-4′,5′-methylenedioxyisoflavone (3), 2′,6,7-trimethoxy-4′,5′-methylenedioxyisoflavone (4), and 6,7-dimethoxy-3′,4′-methylenedioxyisoflavanone (5), all of which had previously been isolated from the extract of the heartwood of the tree; [1] and a further two crystalline compounds.

† Recent permethylation and perdeuteriomethylation studies [6] have shown that flavones with a 5-methoxy-group also show losses of 29 mass units from the molecular ion, and that the 5-OCD_3 group gives rise to prominent M − 30 peaks.

Compound (8) (II; R = OMe), $C_{20}H_{18}O_8$, exhibited i.r. and u.v. absorptions characteristic of an isoflavonoid structure,[1,2] and the n.m.r. spectrum revealed the presence of a methylenedioxy- and four methoxy-groups. Oxidation with potassium permanganate gave 3,4-methylenedioxybenzoic acid, thus placing all four methoxy-groups in ring A. The mass spectrum of compound (8) differed markedly from those of the previously isolated *Cordyla* isoflavonoids in that the M − 15 peak, which is generally of a very low intensity in these and related compounds [1] (Table), is the base peak. This unusual fragmentation pattern was also obtained in the case of compound (9), and is discussed later.

Compound (9) (II; R = H), $C_{19}H_{16}O_7$, showed i.r. and u.v. absorption maxima characteristic of an isoflavonoid structure,[1,2] and the n.m.r. spectrum revealed the presence of a methylenedioxy- and three methoxy-groups. Oxidation of the compound with potassium permanganate gave 3,4-methylenedioxybenzoic acid, thus placing all three methoxy-groups in ring A, and this was confirmed by the mass spectrum which contained peaks at *m/e* 146 and 210 arising from retro-Diels–Alder fission of the heterocyclic ring.

The mass spectral information (Table) reveals that isoflavones which contain a 5-methoxy-group invariably exhibit prominent M − 14 and M − 29 peaks, and the presence of these peaks in the mass spectra of isoflavones appears to be diagnostic of the 5-methoxy-group.† The prominent M − 14 and M − 29 peaks in the mass spectrum of compound (9), therefore, suggest that a 5-methoxy-group is present, and this is confirmed by the chemical shift of the A-ring proton in the compound. The chemical shift of the 5-H in isoflavones and closely related compounds has been shown [1,3–5,7] to be less than τ 2·5, and that of the A-ring proton in compound (9) (τ 3·38) indicates that it is the 6- or 8-H, thus confirming placement of one methoxy-group in the 5-position.

The mass spectrum of compound (9) contains the

Mass spectra * of compounds (7)—(9) and related isoflavones

	$M^{+\cdot}$		M − 14		M − 15		M − 29		M − 31	
Isoflavone	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
6-Hydroxy-2′,7-dimethoxy-4′,5′-methylenedioxy- (7)	342	94							311	100
5,6,7,8-Tetramethoxy-3′,4′-methylenedioxy- (8)	386	80	372	24	371	100	357	43		
5,6,7-Trimethoxy-3′,4′-methylenedioxy- (9)	356	45	342	25	341	100	327	68		
2′,4′,5′,6,7-Pentamethoxy- (1)	372	100			357	18			341	56
3′,4′,6,7-Tetramethoxy- (2)	342	100			327	12				
3′,6,7-Trimethoxy-4′,5′-methylenedioxy- (3)	356	100								
2′,6,7-Trimethoxy-4′,5′-methylenedioxy- (4)	356	100					327	5	325	78
6,7-Dimethoxy-3′,4′-methylenedioxy- (6)	326	100							295	3
5-*O*-Methylgenistein (III)	284	100	270	12	269	4	255	18		
5,7-Dimethoxy-	282	100	268	23	267	18	253	24		
7-Hydroxy-4′,8-dimethoxy-	298	100			283	7				
4′,7,8-Trimethoxy-	312	100			297	5				

* Peaks of less than 2% relative abundance have been omitted.

[5] T. J. Mabry, K. R. Markham, and M. B. Thomas, 'The Systematic Identification of Flavonoids,' Springer-Verlag, New York, 1970, p. 169.

[6] T. J. Mabry, personal communication.

[7] J. S. P. Schwarz, A. I. Cohen, W. D. Ollis, E. A. Kaczka, and L. M. Jackman, *Tetrahedron*, 1964, **20**, 1317.

$M - 15$ peak as the base peak; like compound (8), therefore, this material differs markedly in its mode of fragmentation from compounds (1)—(7), and related isoflavones in which the $M - 15$ peak is of a low intensity (Table). This we attribute to the loss of a methyl group from position 6 in the parent ion, facilitated by steric effects in the highly substituted A ring. This leads to the conclusion that compound (9) is 5,6,7-trimethoxy-3′,4′-methylenedioxyisoflavone (II; R = H). The placing of methoxy-groups in positions 6 and 7 is substantiated by biogenetic considerations, since that oxygenation pattern is found in all the isoflavonoids thus far isolated from *Cordyla africana*.[1]

The absence of prominent $M - 15$ peaks in the mass spectra of less highly substituted 6- and 8-methoxyisoflavones (Table) contrasts with the fragmentations of similarly substituted flavones and flavonols [8] where high intensity $M - 15$ peaks are observed, and are attributed to losses *via ortho*- and *para*-quinonoid intermediates. The reasons for these significant differences in the mass spectral fragmentations of isoflavones, and flavones, and flavonols are not clear.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. U.v. spectra were recorded with a Beckman DB spectrometer for solutions in 95% ethanol. I.r. spectra were recorded with a Unicam SP 200 spectrometer for potassium bromide discs. N.m.r. spectra were recorded with a Perkin-Elmer R12 spectrometer and unless otherwise stated are for solutions in $CDCl_3$ with tetramethylsilane as internal standard. Mass spectra were recorded with an A.E.I. MS9 spectrometer and accurate mass determinations were carried out with an A.E.I. MS 902 spectrometer. Kieselgel GF_{254} silicic acid (Merck) was used for t.l.c.

Extraction of the Heartwood.—By the procedure we have described [9] the heartwood (3·5 kg) was extracted with chloroform, after extraction with light petroleum and diethyl ether. The extract was concentrated under reduced pressure and separated into alkali-soluble and -insoluble portions using aqueous 20% sodium hydroxide. The alkali-soluble portion was obtained as a pale yellow foam (3·0 g), and preparative scale t.l.c. [chloroform–methanol (92 : 8) as solvent] gave a broad band of R_F 0·4—0·7, and several minor bands which have not been further investigated. The major band was scraped from the plate and eluted with acetone; further t.l.c. [chloroform–ethanol (95 : 5) as solvent] gave three bands, the major one of which showed a dark blue u.v. fluorescence. This band was scraped from the plate and eluted with acetone, and the product was crystallised from ethanol to give compound (7) (26·9 mg).

The alkali-insoluble fraction was obtained as an orange gum (8·0 g) which was placed on a 14 × 1 in alumina column, and eluted with chloroform. Two fractions were collected. Fraction (A) after removal of solvent under reduced pressure gave a white solid (3·7 g), and fraction (B) gave an orange oil (2·2 g). A third fraction was eluted from the column with methanol, but this has not been further investigated. Fraction (A) was triturated with methanol, and the methanol-soluble material was separated by t.l.c. [chloroform–benzene–acetone (10 : 10 : 1)] to give compounds (1)—(4). Fraction (B) was subjected to t.l.c. [chloroform–benzene–acetone (5 : 5 : 1)] and three major bands were scraped from the plates. Band 1 (R_F 0·7, blue u.v. fluorescence) gave compound (5). Band 2 (R_F 0·6, greenish u.v. fluorescence) gave compound (8) (14 mg) as pinkish needles from ethanol. Band 3 (R_F 0·5, blue u.v. fluorescence) gave compound (9) (25·5 mg) as colourless needles from methanol.

6-Hydroxy-2′,7-dimethoxy-4′,5′-methylenedioxyisoflavone (7) crystallised from methanol as needles m.p. 252—253° (Found: M^+, 342·0801. $C_{18}H_{14}O_7$ requires M, 342·0798); $\nu_{max.}$ 3430 (OH), 1640 (C=O), 1618, and 1512 (aryl) cm^{-1}; $\lambda_{max.}$ 207, 255, and 312 nm; τ (CD_3OD) 2·10 (1H, s, 2-H), 2·36 (1H, s, 5-H), 3·08 (1H, s, 6′-H), 3·21 (1H, s, 3′-H), 3·40 (1H, s, 8-H), 3·96 (2H, s, O·CH_2·O), 6·00 (3H, s, 7-OMe), and 6·26 (3H, s, 2′-Me).

Compound (7) (5 mg) in methanol was treated with ethereal diazomethane at 0°, and set aside at 0° overnight. Removal of the solvent under reduced pressure followed by crystallisation from methanol gave 2′,6,7-trimethoxy-4′,5′-methylenedioxyisoflavone, m.p. 234—235° identical (m.p., mixed m.p., i.r., t.l.c.) with an authentic sample.

Fusion of compound (7) *with potassium hydroxide.* Compound (7) (5 mg) and solid potassium hydroxide (0·5 g) were fused at 220—230° under nitrogen for 30 min. The pellet was cooled and dissolved in 5N-hydrochloric acid (10 ml), and the solution was extracted with ether (4 × 5 ml). The combined extracts were separated into phenolic and acidic fractions by use of sodium hydrogen carbonate, and the phenolic degradation products were chromatographed alongside standard 4-hydroxy-3-methoxyphenol on Whatman no. 20 paper at room temperature in the solvent systems: (*a*) aqueous 5% sodium formate containing 0·25% formic acid: (*b*) benzene–propionic acid–water (2 : 2 : 1) (organic phase); and (*c*) aqueous 20% potassium chloride. The spots were identified by comparison of the colours produced with diazotised sulphanilic acid, and by their R_F values.

2′,4′,5′,6,7-Pentamethoxyisoflavone (1) crystallised from methanol as needles, m.p. 171—172°; 3′,4′,6,7-tetramethoxyisoflavone (2) crystallised from methanol as broad needles, m.p. 187—188°; 3′,6,7-trimethoxy-4′,5′-methylenedioxyisoflavone (3) crystallised from methanol as needles, m.p. 211—212°; 2′,6,7-trimethoxy-4′,5′-methylenedioxyisoflavone (4) crystallised from methanol as needles, m.p. 234—235°; 6,7-dimethoxy-3′,4′-methylenedioxyisoflavanone (5) crystallised from methanol as fine cubes, m.p. 201—202°; compounds (1)—(5) were identical (m.p., mixed m.p., i.r., t.l.c.) with authentic samples.[1]

5,6,7,8-Tetramethoxy-3′,4′-methylenedioxyisoflavone (8) crystallised from methanol as needles, m.p. 210—212° (Found: M^+, 386·1000. $C_{20}H_{18}O_8$ requires M, 386·1001); $\nu_{max.}$ 1636 (C=O), 1612, and 1520 (aryl) cm^{-1}; $\lambda_{max.}$ 211, 256, and 301 nm; τ 2·35 (1H, s, 2-H), 3·18—3·50 (3H, m, 2′-, 5′-, and 6′-H), 4·16 (2H, s, O·CH_2·O), 6·13br (9H, s, 3 × OMe), and 6·34 (3H, s, OMe).

Oxidation of compound (8) *with potassium permanganate.* Compound (8) (5 mg) and potassium permanganate (50 mg) in acetone (6 ml) were heated under reflux for 2 h. Water (20 ml) was added, and after evaporation of the acetone the solution was saturated with sulphur dioxide and extracted

[8] J. G. Nielsen and J. Moller, *Acta. Chem. Scand.*, 1970, **24**, 2665.

[9] S. H. Harper, A. D. Kemp, W. G. E. Underwood, and R. V. M. Campbell, *J. Chem. Soc.* (*C*), 1969, 1109.

with ether. The acidic components of the product were purified by extraction into sodium hydrogen carbonate, and after recovery by acidification and extraction with ether were run alongside standard 3,4-methylenedioxybenzoic acid on thin-layer plates in the solvent systems: (*a*) benzene–acetic acid (9 : 1); (*b*) dioxan–propionic acid (9 : 1); and (*c*) chloroform–ethanol (9 : 1). The spots were located under u.v. light, and by charring after spraying with sulphuric acid, and shown to be identical.

5,6,7-*Trimethoxy*-3′,4′-*methylenedioxyisoflavone* (9) crystallised from methanol as needles, m.p. 172—173° (Found: M^+, 356·0942. $C_{19}H_{16}O_7$ requires M, 356·0935); $\nu_{max.}$ 1650 (C=O), 1606, and 1515 (aryl) cm^{-1}; $\lambda_{max.}$ 206, 262, and 290sh nm; τ 2·28 (1H, s, 2-H), 2·95—3·20 (3H, m, 2′-, 5′- and 6′-H), 3·38 (1H, s, 8-H), 4·09 (2H, s, O·CH_2·O), 6·08 (6H, s, 2 × OMe), and 6·13 (3H, s, OMe).

Oxidation of compound (9) *with potassium permanganate.* Compound (9) (5 mg) was oxidised with potassium permanganate, and the 3,4-methylenedioxybenzoic acid produced was identified by chromatography as described for compound (8).

Synthesis of 5,7-*Dimethoxyisoflavone.*—2′,4′,6′-*Trihydroxy*-2-*phenylacetophenone.* Phloroglucinol (6·3 g), benzyl cyanide (5·9 g, 7·5 ml), and anhydrous zinc chloride (3·0 g) were stirred together in sodium-dried ether (50 ml) at 0°. Dry hydrogen chloride gas was bubbled through the mixture for 4 h, and the flask was then stoppered and set aside at room temperature overnight. The ether was decanted, and the solid was washed with ether (2 × 10 ml). The solid was then refluxed with water (100 ml) containing conc. hydrochloric acid (2 ml), and the oil obtained on cooling was crystallised from benzene to give 2′,4′,6′-trihydroxy-2-phenylacetophenone (8·6 g) as needles, m.p. 162—164° (lit.,[10] 162°); $\nu_{max.}$ 3380—3200 (OH), 1635 (C=O), 1605, and 1522 (aryl) cm^{-1}; $\lambda_{max.}$ 210, 225, and 291 nm; τ −1·92br (2H, s, 2 × OH), −0·05br (1H, s, OH), 2·76br (5H, s, Ph), 4·22 (2H, s, 3′- and 5′-H), and 5·70 (2H, s, CO·CH_2Ph).

2′-*Hydroxy*-4′,6′-*dimethoxy*-2-*phenylacetophenone.* 2′,4′,6′-Trihydroxy-2-phenylacetophenone (2·4 g) and methyl iodide (3·5 g, 2·5 mol. equiv.) in acetone (100 ml) were refluxed over anhydrous potassium carbonate (4·5 g) for 3 h. Water (250 ml) was added and the mixture was extracted with ether (3 × 50 ml). The combined extracts were dried ($MgSO_4$); removal of the solvent under reduced pressure followed by crystallisation from methanol gave 2′-hydroxy-4′,6′-dimethoxy-2-phenylacetophenone (2·1 g) as buff-coloured platelets, m.p. 114—116° (lit.,[11] 118°); $\nu_{max.}$ 3180 (OH), 1638 (C=O), 1595, and 1508 (aryl) cm^{-1}; $\lambda_{max.}$ 212, 226, and 291 nm; τ −3·78 (1H, s, OH), 2·82br (5H, s, Ph), 4·00 (1H, d, J 3 Hz, 3′- or 5′-H), 4·15 (1H, d, J 3 Hz, 3′- or 5′-H), 5·73 (2H, s, O·CH_2·O), and 6·15—6·30br (6H, s, 2 × OMe).

5,7-*Dimethoxyisoflavone.* 2′-Hydroxy-4′,6′-dimethoxy-2-phenylacetophenone (150 mg) and ethyl formate (3·0 ml) in pyridine (3·0 ml) containing piperidine (5 drops) were refluxed for 5 h. The solution was then poured on crushed ice (*ca.* 100 g) and extracted into ether (3 × 50 ml). The combined extracts were dried ($MgSO_4$), and the ether was removed under reduced pressure. 5,7-Dimethoxyisoflavone (83 mg) was obtained as plates (from methanol), m.p. 109—111° (lit.,[11] 112°); $\nu_{max.}$ 1648 (C=O), 1612, and 1500 (aryl) cm^{-1}; $\lambda_{max.}$ 210, 253, and 306sh nm; τ 2·28 (1H, s, 2-H), 2·38—2·90 (5H, m, Ph), 3·58—3·70 (2H, m, 6- and 8-H), 6·14 (3H, s, OMe), and 6·22 (3H, s, OMe).

We thank the Director of the National Chemical Research Laboratory, C.S.I.R., Pretoria, South Africa, and Dr. R. H. Hall for the recording of mass spectra. We are grateful to Dr. L. Jurd for the samples of 7-hydroxy-4′,8-dimethoxy- and 4′,7,8-trimethoxy-isoflavone, and Dr. J. B. Harbone for mass spectral information for 5-*O*-methylgenistein. We also thank Dr. R. J. Phelps, Division of Biological Sciences of this University, for assistance in obtaining wood samples, and Professor S. H. Harper of this University for facilities which have enabled this investigation to be completed.

[3/480 *Received*, 5*th March*, 1973]

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