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DERRUGENIN, A NEW ISOFLAVONE FROM *DERRIS ROBUSTA* SEED SHELLS

S. S. CHIBBER and R. P. SHARMA
Department of Chemistry, University of Delhi, Delhi-110007, India

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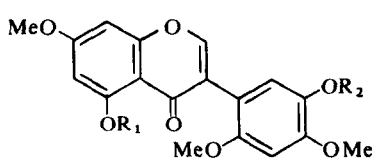
Key Word Index—*Derris robusta*; Leguminosae; seed shells; derrugenin; 5,5'-dihydroxyi-7,2',4'-trimethoxy-isoflavone.

In continuation [1] of our work on the seed shells of *D. robusta*, we wish to report the isolation of a new isoflavone, derrugenin, from its benzene extract. It analysed for $C_{18}H_{16}O_7$ (M^+, 344). UV and IR spectra suggested a 5-hydroxyisoflavone skeleton. Since the compound was insoluble in $CDCl_3$, NMR of its acetate was recorded, which showed the presence of two phenolic acetoxyls at δ 2.30 and 2.37, three singlets at 3.69, 3.77 and 3.88 due to three methoxyls and one proton singlet at 7.80 corresponding to H-2 of the isoflavone. The bathochromic shift [2] of 10 nm with $AlCl_3$, the absence of a bathochromic shift [2] with NaOAc in UV and two doublets (J = 2.5 Hz) centred at δ 6.77 and 6.59 corresponding to H-8 and H-6, respectively, suggested 5-hydroxy-7-methoxy substitution for ring A. The remaining two methoxyls and one hydroxyl are therefore located in ring B. Presence of a M − 31 peak in the mass spectrum of the compound indicated that one methoxyl was located at the 2'-position [3, 4]. Sharp singlets at δ 6.95 and 6.69, each integrating for one proton, in the NMR of derrugenin acetate were attributed to *para*-oriented 6'- and 3'-protons, respectively, indicating a 2',4',5'-substitution pattern in the side phenyl ring, which was further substantiated by conversion of derrugenin into robustigenin [1] (**3**) and robustigenin 5-*O*-methyl ether (**4**) by methylation. Whether the hydroxyl is at the 4'- or 5'-position in derrugenin was established by permanganate oxidation [5] of its diethyl ether (**6**), when 2,4-dimethoxy-5-ethoxybenzoic acid was obtained. The latter was identified by direct comparison with an authentic sample prepared by the method of Rajagopalan *et al.* [6]. Prominent peaks at *m/e* 167 and 177 (arising from retro-Diels–Alder fission of the heterocyclic ring) in the mass spectrum of derrugenin, further supported the above assignments. Hence derrugenin is 5,5'-dihydroxy-7,2',4'-trimethoxyisoflavone (**1**).



1 $R_1 = R_2 = H$
2 $R_1 = R_2 = Ac$
3 $R_1 = H$; $R_2 = Me$
4 $R_1 = R_2 = Me$
5 $R_1 = H$; $R_2 = Et$
6 $R_1 = R_2 = Et$

EXPERIMENTAL

^1H NMR spectra were taken at 60 MHz (unless otherwise stated) in $CDCl_3$ and chemical shifts are given in δ (ppm) scale relative to TMS; UV spectra were obtained in MeOH and IR spectra as KBr discs.

Isolation. Air-dried and coarsely powdered seed shells (750 g) of *D. robusta* were defatted with hot petrol (60–80°) and then extracted with hot C_6H_6 (4 × 500 ml). The C_6H_6 extract was concd and subjected to column chromatography over Si gel using C_6H_6–EtOAc (9.7:0.3) as the eluent, when a crystalline compound (800 mg), derrugenin (**1**), was obtained. TLC: R_f 0.40 (C_6H_6–EtOAc, 9:1); mp 218–19°; λ_{max}^{MeOH} nm: 260, 315sh, +NaOAc 260, 315sh; +$AlCl_3$: 270, 312, 365. ν_{max}^{KBr} cm^{-1}: 3450, 1645, 1605, 1042 and 824. MS (*m/e*, %): 344 (100), 329 (M − 15, 32), 313 (M − 31, 56), 177 (17) and 167 (36) (RDA fragments).

Acetylation of derrugenin (50 mg) with Py (3 ml) and Ac_2O (3 ml) gave a diacetate (**2**) as needles (40 mg), mp 230–31°; ν_{max}^{KBr} cm^{-1} 1754, 1639, 1618, 1036, 903 and 825. ^1H NMR ($CDCl_3$): δ 2.30, 2.37 (2 × 3H, each *s*, 2 × O—C(=O)—C$\underline{H}_3$), 3.69 (3H, *s*, OC$\underline{H}_3$), 3.77 (3H, *s*, OC$\underline{H}_3$), 3.88 (3H, *s*, OC$\underline{H}_3$), 6.59 (1H, *d*, J = 2.5 Hz, Ar—$\underline{H}_6$), 6.69 (1H, *s*, Ar—$\underline{H}_{3'}$), 6.77 (1H, *d*, J = 2.5 Hz, Ar—$\underline{H}_8$), 6.95 (1H, *s*, Ar—$\underline{H}_{6'}$) and 7.80 (1H, *s*, $\underline{H}_2$).

Methylation of derrugenin (150 mg) by Me_2CO–K_2CO_3–Me_2SO_4 gave a mixture of 2 compounds which were characterized [1] as robustigenin **3** (20 mg) and robustigenin-5-*O*-methyl ether **4** (110 mg), respectively, by direct comparison (mp, mmp, co-TLC and co-IR) with authentic samples.

Ethylation of derrugenin (150 mg) by Me_2CO–K_2CO_3–Et_2SO_4 gave a mixture of 2 compounds **5** (25 mg) and **6** (100 mg). Compound **5** mp 136–37°; λ_{max}^{MeOH} nm: 260, 298sh; +NaOAc 260, 295sh; +$AlCl_3$ 270, 305; +NaOMe 270, 295, 336; ν_{max}^{KBr} cm^{-1}: 1645, 1613, 1040 and 819. ^1H NMR (90 MHz, $CDCl_3$): δ 1.44–1.59 (3H, *t*, J = 7 Hz, —OCH$_2$—C$\underline{H}_3$), 3.80 (3H, *s*, OC$\underline{H}_3$), 3.89 (6H, *s*, 2 × OC$\underline{H}_3$), 4.03–4.33 (2H, *q*, J = 7 Hz, O—C$\underline{H}_2$—CH$_3$), 6.40 (2H, *s*, Ar—$\underline{H}_6$ and Ar—$\underline{H}_8$), 6.65 (1H, *s*, Ar—$\underline{H}_{3'}$), 6.92 (1H, *s*, Ar—$\underline{H}_{6'}$), 7.87 (1H, *s*, $\underline{H}$-2) and 13.35 (1H, *s*, chelated hydroxyl). Compound **6**, mp 144–45°; λ_{max}^{MeOH} nm: 260, 295sh: ν_{max}^{KBr} cm^{-1}: 1642, 1605, 1031, 883 and 810. ^1H NMR ($CDCl_3$): δ 1.16–1.65 (2 × 3H, 2 *t*, 2 × OCH$_2$—C$\underline{H}_3$), 3.56 (3H, *s*, OC$\underline{H}_3$), 3.73 (6H, *s*, 2 × OC$\underline{H}_3$), 3.74–4.30 (4H, *m*, 2 × OC$\underline{H}_2$—CH$_3$), 6.18 (1H, *d*, J = 2.5 Hz, Ar—$\underline{H}_6$), 6.24 (1H, *d*, J = 2.5 Hz, Ar—$\underline{H}_8$), 6.43 (1H, *s*, Ar—$\underline{H}_{3'}$), 6.80 (1H, *s*, Ar—$\underline{H}_{6'}$) and 7.56 (1H, *s*, $\underline{H}$-2). On the basis of the above data, **5** and **6** were characterized as derrugenin-5'-*O*-ethyl- and 5,5'-di-*O*-ethyl ethers, respectively.

Oxidation [5] *of derrugenin diethyl ether* (100 mg) in Me_2CO (50 ml) with $KMnO_4$ (10 × 20 mg) gave 20 mg of a slightly yellow solid which, when subjected to column chromatography over Si gel using $CHCl_3$–MeOH (9.9:0.1) as the eluent, gave a colourless crystalline solid (15 mg) mp 134–36°, identified as

2,4-dimethoxy-5-ethoxybenzoic acid by direct comparison (mp, mmp, co-TLC and co-IR) with a synthetic sample.

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ALKALOIDS FROM LEAVES OF *ANNONA SQUAMOSA*

P. K. Bhaumik*, B. Mukherjee†, J. P. Juneau‡, N. S. Bhacca‡ and R. Mukherjee§

* Department of Chemistry, Krishnagar College, West Bengal, India; † Department of Pharmacology, B. C. Roy Post-Graduate Institute of Basic Medical Sciences, Calcutta-700020, India; ‡ Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, U.S.A. and § Faculdade de Farmacia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

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Key Word Index—*Annona squamosa*; Annonaceae; alkaloids; (−)-xylopine; (+)-*O*-methylarmepavine; lanuginosine.

INTRODUCTION

Annona squamosa L. has been in use in folk medicine [1] for quite some time and an EtOH extract of the leaves and stems is reported to have anti-cancer activity [2]. Isolation of a number of alkaloids [3, 4], terpene derivatives [5] and a novel diazepine, squamolone [6] from this plant has been reported. However, detailed chemical and pharmacological investigations on the leaves are still awaited. Recent pharmacological screening on the total bases from the leaves revealed a strong acetylcholine-like activity[‖] which prompted us to undertake a complete chemical investigation of the crude extract. This resulted in the isolation of friedelin and the alkaloids (−)-xylopine, (+)-*O*-methylarmepavine and lanuginosine for the first time from this source.

‖ Preliminary pharmacological investigations have been carried out in the Department of Pharmacology, B. C. Roy Post-Graduate Institute of Basic Medical Sciences, Calcutta 700020, India.

RESULTS AND DISCUSSION

The compounds were isolated by solvent extraction, chromatography over Brockmann alumina and subsequent purification of different fractions.

The first alkaloid **1**, $C_{18}H_{17}NO_3$ (M^+ 295), mp 123°, showed UV absorption and MS fragmentation pattern typical of aporphine alkaloids [7]. The ^1H NMR spectrum, with stepwise irradiation of selective absorptions and the use of nuclear Overhauser effect (NOE) (Table 1) was in agreement with structure **2**, providing additional means for assigning the substituents. Direct comparison with an authentic sample revealed its identity with (−)-xylopine [8, 9].

The polar liquid alkaloid **2**, $C_{20}H_{25}NO_3$ (M^+ 327),

Table 1. ^1H NMR spectral analysis of (−)-xylopine

Chemical shift δ	Appearance of signal and other observations	Assignment and other conclusions	
6.83 (A)	An ABX pattern (J_{AB} = 2.5 Hz (*meta*), J_{AX} =	A → H-10	(line broadening in X was ob-
6.83 (B)	8.5 Hz (*ortho*), J_{BX} = 0 Hz (*para*)), A and B adja-	B → H-8	served because of overlap of A
7.88 (X)	cent to OMe inferred because of their high field positions. Low field position of H-11 is typical of the aporphines [10].	X → H-11	and B resonances)
3.78 (M_3)	Three-proton singlet, when M_3 irradiated, A and B exhibit 15–20% NOE enhancements.	M_3 → OMe	(so OMe is at C-9)
6.54 (E)	One proton singlet in aromatic region, exhibits (~25%) NOE enhancements when benzylic methylene resonance region is irradiated.	E → H-3	
5.92 (M)	A two-proton four-line pattern (J_{MN} = 1.5 Hz)	M → H-12	
6.05 (N)	representing methylenedioxy group protons [10].	N → H-12	
3.70 (T)	A wide (18 Hz) one proton pattern exhibiting splittings of 12 and 6 Hz.	T → H-6_a	(the proton has very likely an axial orientation)