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ISOFLAVONOIDS FROM *TAVERNIERA ABYSSINICA*

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ABSTRACT: From the roots of *Taverniera abyssinica* (Leguminosae),a medicinal plant sold in many markets of central Ethiopia under the name of 'Dingetegna', four isoflavonoid derivatives have been isolated. One of these was identified as 3,4-Dihydroxy-9-methoxypterocarpan which appears to be a new natural product, while the others were the known isoflavonoids formononetin, afrormosin and the known pterocarpan medicarpin.

INTRODUCTION

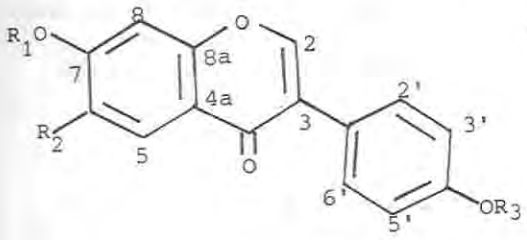
A survey of 19 medicinal plant markets of central Ethiopia conducted by Kloos et al (1) showed that a significant number of vendors were selling the roots of a plant known in Amharic as "Dingetegna", literally meaning "medicine for sudden illness", and used for the treatment of headache, stomach ache and fever. It is commonly administered by chewing the roots and swallowing the juice. Kloos et al were unable to determine the botanical identity of "Dingetegna".

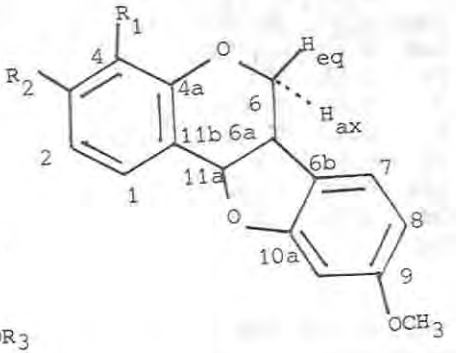
Using field notes of botanists from the National Herbarium, Addis Ababa University, Ethiopia, we were able to locate flowering "Dingetegna" plants 52 km west of Addis Ababa on the Butajira road near Melka Konture at an altitude of 2150 m. The plant was unequivocally identified as the legume *Taverniera abyssinica* A. Rich. According to Thulin (2), this species belongs to a small genus, *Taverniera*, which comprises only 15 species and is found in North East Africa and S. West Asia. *T.abyssinica* is not known to occur elsewhere and even in Ethiopia, it is confined to the provinces of Shoa and Tigrai.

A literature search on the genus *Taverniera* failed to provide reports on either the chemistry or the pharmacology of any of its species except for a recent chemical screening for *T. aegyptica* Boiss., which indicated the presence of alkaloids and coumarins (3). Because of the importance of the roots of *T.abyssinica* in Ethiopian traditional medicine we undertook to study its chemical as well as pharmacological aspects and we report here the results of our chemical investigations.

RESULTS AND DISCUSSION

Chromatographic separations of the petroleum ether as well as chloroform extracts of the roots of *T. abyssinica* led to the isolation of four compounds.





1 $R_1 = R_2 = H$, $R_3 = CH_3$

2 $R_1 = CH_3$, $R_2 = R_3 = H$

3 $R_1 = H$, $R_2 = OCH_3$, $R_3 = CH_3$

4 $R_1 = H$, $R_2 = OH$

5 $R_1 = H$, $R_2 = OAc$

6 $R_1 = R_2 = OH$

7 $R_1 = R_2 = OAC$

Compound 1 was identified as the well known isoflavonoid, formononetin, based on comparison of the spectroscopic data including ^{13}C NMR with literature reports (4). However since the ^{13}C NMR spectrum of the isomeric compound (2) (5) is indistinguishable from that of formononetin (1), we performed NOE experiments which enabled us to prove the spatial proximity of H-3' and H-5' with the methoxy group.

Compounds 3 and 4 were readily identified as the isoflavonoid afrormosin and the pterocarpan medicarpin respectively, based on comparison of their spectroscopic data with those given in the literature (6, 7, 8).

Interestingly, the other pterocarpan isolated from this plant was characterized as 3,4-dihydroxy-9-methoxypterocarpan (6), a compound first reported by Ingham et al (9) as a fungal-mediated transformation product of medicarpin (4). Hence this is the first report of compound 6 as a natural product. Furthermore characterization of this compound by these workers (9) was based on the Gibbs test, MS and UV data as well as derivatization to the corresponding trimethyl ether. They also remarked that the available data did not enable them to unequivocally distinguish the fungal transformation product 6 from the isomer that would place the methoxyl at position 3.

Table 1: ^{1}H NMR spectral data* of compounds 4-6

H	4	5	6
1	7.37 d (7.8)	7.51 d (8.7)	6.98 d (8.3)
2	6.53 dd (2.3, 7.8)	6.78 dd (2.4, 8.2)	6.67 d (8.3)
4	6.39 d (2.3)	6.69 d (2.4)	-
6eq.	4.21 dd (4.5, 10)	4.25 dd (4.4,10)	4.31 dd(4.8,10)
6ax.	3.60 t (10)	3.61 t (10)	3.66 t (10)
6a	3.51 ddd	3.56 ddd	3.56 ddd
7	7.11 d (8.4)	7.11 d (8.7)	7.11 d (8.3)
8	6.43 dd (2.3, 8.4)	6.43 dd(2.4,8.7)	6.43 dd(2.5,8.3)
10	6.42 brs	6.42 brs	6.42 brs
11a	5.48 d (6.7)	5.50 d (6.6)	5.51 d (6.9)
OMe	3.75	3.75	3.75
OAc	-	2.28	-

*Run at 400 MHz in $CDCl_3$ with chloroform signal as internal standard, values in ppm (δ), coupling constants (Hz) in parentheses.

Table 2: ^{13}C NMR data of compounds 4, 6 and 7 in $CDCl_3$, (ppm) at 100.6 MHz

C-No.	4	6	7
1	132.2	121.8	128.0
2	109.7	109.5	115.8
3	157.0*	144.4*	143.4
4	103.7	131.5	+
4a	156.7*	143.1*	148.7
6	66.5	67.0	66.9
6a	39.5	39.7	39.4
6b	119.1	118.6	118.4
7	124.7	124.7	124.8
8	106.4	106.5	106.7
9	161.1*	161.2*	161.3*
10	96.9*	97.0*	96.9
10a	160.7*	160.5*	160.7*
11a	78.5	78.4	77.7
11b	112.7	+	119.4
OCH_3	55.5	55.5	55.5
C=O(OAc)	-	-	168.0,168.5
Me(OAc)	-	-	20.3, 20.6

*: may be interchanged; + : not observed

HRMS of 6 gave a parent ion at $C_{16}H_{14}O_5$. Its ^{1}H NMR spectrum (see Table 1) indicated a close similarity between chemical shift values for all of its protons with those of medicarpin (4) except

for the disappearance of the signal of H-4 and shifts in the resonances of the two aromatic protons H-1 and H-2. This was indicative of the presence of an additional hydroxy group. That the two hydroxy groups should be placed at either 1,2 or 3,4 positions was deduced from the fact that the two aromatic protons are ortho to each other. The 3,4-dihydroxy position was proved by ^{1}H-^{1}H COSY experiment (Fig. 1) which showed a correlation peak indicating that H-1 and H-11a are undergoing a spin-spin coupling interaction. Furthermore in the ^{13}C spectrum the occurrence in the A ring of the oxygenated quaternary carbons at 144.4, 143.1 and 131.5 requires that they be in a pyrogallol substitution pattern as in 6.

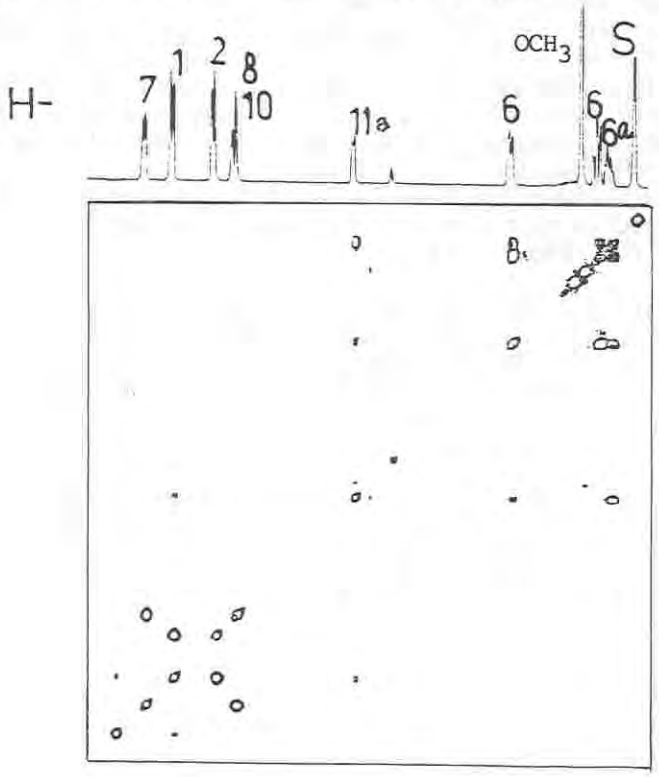


Fig. 1. ^{1}H-^{1}H COSY spectrum of compound **6**

Comparison by TLC of the extracts of the plant material purchased from the market with that collected from the field revealed identical constituents.

EXPERIMENTAL

General: Mps were measured on Kofler hot stage and are uncorrected. NMR spectra were measured on a Bruker AM-400 spectrometer in $CDCl_3$ and CD_3OD at 400 MHz (^{1}H) and 100.6 MHz (^{13}C). MS were recorded at 70 eV.

TLC was performed on silica-gel 60, Merck, using pet. ether EtOAc, 4:1 (solvent system 1) and column chromatography was carried out on Sephadex LH-20 using $CHCl_3$ /MeOH,1:1 (solvent system 2), and silica-gel 60 Merck using gradient elution with pet. ether and EtOAc (solvent system 3).

Plant material: The roots of *T. abyssinica* A. Rich [Leguminosae] were purchased from the market in Addis Ababa and also collected from the locality mentioned in the text. A herbarium specimen was deposited in the National Herbarium, Addis Ababa University under the cipher Mesfin T. 3687 (Eth).

Extraction and isolation: The powdered roots (400 g) from the market sample were extracted with petroleum ether and chloroform successively using Soxhlet apparatus. The petroleum ether extract was concentrated in vacuo to give an oily residue, which was chromatographed on Sephadex LH 20; 9 fractions, each 50 ml, were collected and analyzed by TLC using solvent system 1. Fraction 6 contained mainly compound **4** and was further purified by silica gel cc using solvent system 3 (10% EtOAc).

The crude chloroform extract was subjected to cc over silica gel and eluted with solvent system 3 and yielded compounds 3 (15% EtOAc), 6 (18%), and 1 (20%).

Formononetin (1). Mp 255-258°C (from PhH/EtOAc). UV λ_{max} (MeOH) nm: 247, 302. MS m/z (rel. int.): 268 (M^+, 100) 267 (37), 151 (20), 132 (35). ^{1}H NMR ($CDCl_3$ -CD_3OD, 1:1): δ 3.79 (s, OMe), 7.72 (s, H-2), 7.82 (d, J = 8.7 Hz, H-5), 6.68 (dd, J = 2.3, 8.7 Hz, H-6), 6.60 (d, J = 2.3, H-8), 7.20 (H-2', H-6'), 6.72 (H-3', H-5'). ^{13}C-NMR identical to literature (4).

Afrormosin (3). Mp 225 - 228°C (PhH/EtOAc). MS m/z (rel. int.): 298 (M^+), 283, 267, 255, 166 (19). ^{1}H NMR ($CDCl_3$-CD_3OD,1:1): δ 3.8 (s, OMe-6), 3.66 (s, OMe-4'), 7.78 (s, H-2), 7.39 (s, H-5), 6.75 (s, H-8), 7.28 (H-2', H-6'), 6.79 (H-3', H-5'). ^{13}C same as lit. (6).

Medicarpin (4). Mp 125-127°C (PhH). $[\alpha]_D$ = - 230° (c = 0.1, MeOH) (lit. (8) mp 127-128°C, $[\alpha]_D$ = - 234°. MS m/z (rel. int.): 270 (M^+, 100), 255, 161, 148. ^{1}H NMR and ^{13}C NMR see Tables 1 and 2. Compound **4** yielded by the usual methods acetate 5 mp 119-121°C, for ^{1}H NMR data see Table 1.

3,4-Dihydroxy-9-methoxypterocarpan (6). Mp 168-170°C. $[\alpha]_D$ = -5° (c = 0.2 MeOH). IR, υ_{max} (KBr) cm^{-1} : 3350-3250 (br), 2950, 1610, 1460, 1340. MS m/z (rel. int.): 286 (M^+ 100), 271 (32), 269. ^{1}H NMR and ^{13}C NMR see Tables 1 and 2. This compound also yielded the diacetate (7): δ ^{1}H NMR ($CDCl_3$): 7.42 (d, J = 8.7 Hz, H-1), 6.87 (d, J =8.7 Hz, H-2), 5.51 (d, J =5.5, H-11a), 7.10 (d, J =8.7, H-7), 6.43 (dd, J = 2.4, 8.7 Hz, H-8), 6.42 (brs, H-10), 3.75 (OMe), 2.28, 2.27 (2 x OAc). ^{13}C NMR see Table 2.

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REFERENCES

1. H. Kloos, A. Tekle, L.W. Yohannes, A. Yosef, A. Lemma, Ethiop. Med. J., 16, 33(1978).
2. a. M. Thulin, "The Leguminosae of Ethiopia," Opera Botanica, No. 63, Sweden (1983).
   b. M. Thulin, "The Revision of *Tavernaiera* (Leguminosae-Papilionoideae," Act. Univ. Ups. Symb. Bot. Ups., XXV:1, II, 51 pp, Uppsala, Sweden.
3. A. M. Rizk, H.I. Heiba, H.A. Ma'ayergi, K.H. Batanouny, Fitoterapia, LVII, 3(1986).
4. J. B. Harborne and T. J. Mabry, "The Flavonoids: Advances in Research", Chapman and Hall, New York, pp. 19-134(1982).
5. E. Wenkert and H. Gottlieb, Phytochemistry, 16, 1811(1977).
6. H. C. Jha, F. Zilliken, E. Breitmaeir, Canad. J. Chem., 58, 1211(1980).
7. T.B.H. McMurry, E. Martin, D.M.X. Donnelly and J.C. Thompson, Phytochemistry, 11, 3283(1972).
8. P. Lebreton, K.R. Markham, W.T. Swift, Oung-Boran, T.J. Mabry, Phytochemistry, 6, 1675(1967).
9. J.L. Ingham, Phytochemistry, 15, 1489(1967).
10. T.A. Geissman, "The Chemistry of Flavonoid Compounds", MacMillan, New York, p. 72(1962).