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SOME ALKALOIDS FROM *AEGLE MARMELOS*

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Key Word Index—*Aegle marmelos*; Rutaceae; alkaloids; cinnamic acid amides.

Abstract—From dry leaves of *Aegle marmelos*, four new alkaloids, *N*-2-[4-(3′, 3′-dimethylallyloxy)phenyl]ethyl cinnamide, *N*-2-hydroxy-2-[4-(3′, 3′-dimethylallyloxy) phenyl]ethyl cinnamide, *N*-4-methoxystyryl cinnamide and *N*-2-hydroxy-2-(4-hydroxyphenyl)ethyl cinnamide were isolated and characterized. Also isolated were aegeline and a purple compound whose structure has not yet been established.

INTRODUCTION

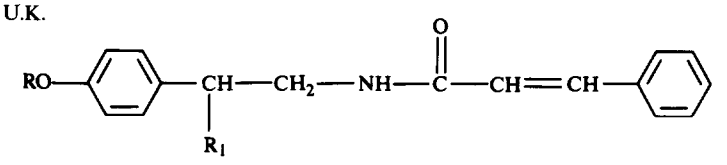
A report* that the leaves of *Aegle marmelos* (Rutaceae) were being used in Bangladesh for fertility control and that the active principles were steroidal hormones led us to re-examine this much investigated plant [1]. However, although we could not obtain any proof for the presence of hormonal steroids in the leaves, we isolated four new alkaloids of the aegeline type [2, 3]. A recent publication reporting the isolation and structure determination of one of these compounds, marmeline (**2**) [4] from unripe fruits, prompts us to publish our results.

* Personal communication from Professor D. Venkatesan, IISc, Bangalore, India, on information given by Professor D. Hodgkin, Cambridge, U.K.

RESULTS AND DISCUSSION

The neutral fraction of the ethyl acetate extract of the dry leaves of *A. marmelos* was partitioned between aqueous methanol and *n*-hexane and the methanol-soluble fraction subjected to CC on Sephadex LH-20 and Si gel to yield alkaloids **1**–**4**, aegeline (**5**) and an alkaloid (**6**). The structures of these compounds were determined entirely by spectral methods and the data is presented in Tables 1 and 2.

Based on the data presented in Tables 1 and 2, alkaloids **1**, **2**, **3**, and **4** have been assigned the following structures:



1 R = —CH_2—CH = CMe_2, R_1 = H

2 R = —CH_2—CH = CMe_2, R_1 = OH

4 R = H, R_1 = OH

5 R = Me, R_1 = OH

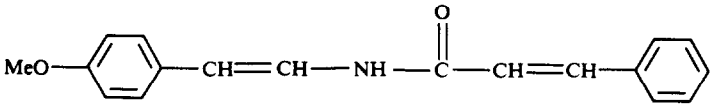


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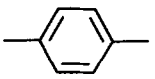
Table 1. Selected mass spectral, UV and IR data of compounds **1**–**5**

Compound	Mass M^+	UV λ nm (log ε)	IR ν cm^{-1}
1	—	218(4.48), 225(4.48), 277(4.56)	3284, 1648, 1612
2	351	218.5(4.40), 224(4.41), 276(4.43)	3280, 1660, 1620*
3	279	290(4.30), 305(4.25), 345(4.35)	3440, 1670, 1658, 1638, 1610, 1602
4	283	218(4.38), 224(4.38), 276(4.49)	3260, 1645, 1610
5	297	218, 225, 275	3260, 1660, 1620

*We do not observe the bands at 1730 and 1725 cm^{-1} reported in ref. [4].

Table 2. ^{1}H NMR data of compounds **1–5** (δ-values with TMS as int. standard)

	1	2	3	4	5
$Me_2C=$	1.70 *s* 1.77 *s*	1.70 *s* 1.74 *s*	—	—	—
=CH–	5.47 *m*	5.43 *m*	—	—	—
$-OCH_2-$	4.47 *d* *J* = 6 Hz	4.46 *d* *J* = 7 Hz	—	—	—
MeO–	—	—	3.78 *s*	—	3.79 *s*
	6.84 *d* 7.11 *d* *J* = 8 Hz	6.94 *d** *J* = 8 Hz	6.81 *d** *J* = 8 Hz	6.83 *d* 7.23 *d* *J* = 8 Hz	6.88 *d** *J* = 8 Hz
Benzylic CH_2/CH	2.8 *t* *J* = 7 Hz	4.81 *dd* *J* = 9 and 3 Hz	—	4.72 *dd* *J* = 9 and 3 Hz	4.70 *dd* *J* = 9 and 3 Hz
$-CH_2-N$	3.6 *m*	3.09–4.04 *m*	—	3.03–3.88 *m*	3.44 *m*
–CH=CH–	6.3 *d* 7.6 *d* *J* = 16 Hz	6.44 *d* 7.68 *d* *J* = 16 Hz	6.22 *d** *J* = 15 Hz 6.65 *d* 7.7 *d* *J* = 16 Hz	6.55 *d* 7.59 *d* *J* = 16 Hz	6.55 *d* 7.60 *d* *J* = 16 Hz
Phenyl	7.23–7.57 *m*	7.19–7.59 *m*	7.2–7.77 *m*	7.23–7.51 *m*	7.14–7.63 *m*

*No definite assignment could be made for the low field portion of the 1,4-disubstituted phenyl/olefinic AB quartets as it falls within the multiplet due to the phenyl ring.

The alkaloids **1–4** are *trans*-cinnamide derivatives, as seen from UV data and the *trans*-coupling constants (J = 16 Hz) of the olefinic protons of the cinnamoyl group.

The *trans* nature of the styryl double bond in **3** is shown by the large coupling constant (J = 15 Hz) of the one-proton doublet at δ 6.22. Structure **3** was confirmed by dehydration of aegeline (**5**) using tosyl chloride in pyridine to give a product identical (TLC, mp, mmp and IR) with **3**.

The structure of alkaloid **4** was confirmed by synthesis. Condensation of β-hydroxy-β-(4-hydroxyphenyl)ethylamine [5] with cinnamoyl chloride gave a product identical (mass, UV and TLC) with **4**. The IR spectrum could not be run in solution due to its insolubility in chloroform. The potassium bromide spectrum exhibited minor differences.

A purple compound, **6**, was also isolated after repeated flash chromatography on Si gel for which no structure assignment has been made. The spectral data of **6** are recorded in the Experimental. It was not possible to obtain it completely pure and further work is in progress to elucidate the structure.

EXPERIMENTAL

Mps are uncorr. IR spectra were recorded as KBr discs and UV spectra in 95% EtOH. NMR spectra were run at 90 MHz in $CDCl_3$ with the addition of one or two drops of DMSO-d_6, except for that of **1** taken in $CDCl_3$. TMS was used as int. standard.

Si gel refers to 70–325 mesh and for flash chromatography finer than 200 mesh.

Dry powdered leaves of *A. marmelos* Corr. (5.4 kg) after extraction with *n*-hexane (9 l. and 5 l. × 2) were extracted with EtOAc (6.5 l. and 5 l. × 2) and the solvent removed. The crude residue was redissolved in EtOAc (1.1 l.), extracted at 0° with 3 M HCl, washed with satd NaCl soln, then extracted with cold 10% Na_2CO_3 soln and the neutral extract washed with satd NaCl soln, dried (Na_2SO_4) and evaporated to leave a residue. The neutral extract was partitioned between MeOH and *n*-hexane. It was dissolved in MeOH–H_2O (9:1; 500 ml) and extracted with *n*-hexane (5 × 250 ml). The combined hexane extracts were washed with satd NaCl soln, dried (Na_2SO_4) and evaporated to leave the hexane-soluble portion. The MeOH layer was evaporated, the residue extracted with EtOAc, washed with satd NaCl soln, dried (Na_2SO_4) and evaporated to leave the MeOH-soluble portion of the neutral extract (40.4 g). This was chromatographed on Sephadex LH-20 using CH_2Cl_2–hexane (6:1), CH_2Cl_2–Me_2CO (6:1), CH_2Cl_2–Me_2CO (4:1), CH_2Cl_2–Me_2CO (1:1) and finally Me_2CO. Fractions were collected and monitored by TLC and rechromatographed on Si gel.

Isolation of alkaloids **1–3**. From the CH_2Cl_2–hexane (6:1) eluate were isolated the following. Alkaloid **1**: colourless crystals (60 mg), mp 125–126° (MeOH). (Found: C, 79.10; H, 7.62; N, 4.195. $C_{22}H_{25}NO_2$ requires: C, 78.77; H, 7.51; N, 4.18%.) Alkaloid **2**: colourless needles (80 mg) mp 159–161° (MeOH) M^+ 351. (Found: C, 75.12; H, 7.26; N, 3.87. $C_{22}H_{25}NO_3$ requires: C, 75.18; H, 7.17; N, 3.99%.) Alkaloid **3**: yellow needles (25 mg) mp 191° (MeOH) M^+ 279. (Found: C, 77.40; H, 6.09; N, 4.92. $C_{18}H_{17}NO_2$ requires: C, 77.39; H, 6.13; N, 5.0%.)

Dehydration of aegeline (**5**). Aegeline (27 mg) in pyridine (1 ml) was treated at 0° with *p*-toluene sulphonyl chloride (19 mg), stirred for 5 min and the reaction mixture allowed to warm to room temp. and then stirred at 70° for 4 hr. The pyridine was removed under red. pres. and the residue dissolved in CH_2Cl_2, washed with 3 M HCl, H_2O, 10% Na_2CO_3 and H_2O, and dried (Na_2SO_4) and evaporated. The residue was chromatographed on Si gel using CH_2Cl_2–*n*-hexane (4:1) to obtain a yellow solid which spontaneously crystallized, mp 192–193°. This was identical (TLC, UV, IR in KBr, mmp) to **3**.

Aegeline (**5**). Colourless needles, (1.5 g) mp 172–176° (MeOH). M^+ 297, $C_{18}H_{19}NO_3$.

Alkaloid **6**. Purple crystals (40 mg), mp 167° (MeOH). UV λ_{max} nm: 202, 220, 225, 276, 283 (sh), 358 and 408. IR ν_{max} cm^{-1}: 3430, 2958, 2920, 2860, 1740, 1700, 1600, 1522, 1446, 1395, 1340,

1302, 1230, 1170, 1130, 1114, 1075, 1060, 980, 897, 807, 780, 728, 705, 670 and 610. ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 1.28*s*, 1.52*t*, 1.78*d*, 3.0*s*, 3.3*s*, 3.55*s*, 3.78*s*, 4.38*m*, 4.8*dd*, 5.18*m*, 6.1*d*, 6.18, 6.32, 6.5 (*d*, J = 16 Hz), 6.85 (*d*, J = 9 Hz), 7.1–7.9*m*, 8.56*s*, 9.14*s*, 9.28*s*.

Isolation of alkaloid **4**. From the CH_2Cl_2–Me_2CO (1 : 1) eluate was isolated alkaloid **4**: colourless needles (20 mg), mp 158° melts, solidifies and remelts 228–231° (MeOH) M^+ 283. (Found: C, 71:69; H, 6.4; N, 5.90. $C_{17}H_{17}NO_3$ requires: C, 72.06; H, 6.05; N, 4.94%.)

Synthesis of N-2-*hydroxy*-2-(4-*hydroxyphenyl*)*ethylcinnamide* (**4**). To β-hydroxy-β-(4-hydroxyphenyl)ethylamine [5] (210 mg) in 10% aq. NaOH soln (0.7 ml) was added powdered cinnamoyl chloride (240 mg) in a stoppered flask and this was then shaken vigorously for 15 min. MeOH was added to the reaction mixture, which was evaporated and the solid filtered off. The mother liquor was evaporated and chromatographed on Si gel to obtain the amide which recrystallized from MeOH–Et_2O, mp 177°. The UV, TLC and MS of this product were identical to **4**. The IR, which could be measured only in KBr, showed minor differences, mmp 172° and 222–224°.

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