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A NEW BISCOUMARIN AND OTHER CONSTITUENTS FROM *Guazuma tomentosa* ROOTS

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*A new dicoumarol, 3,3′-methylenebis(4,6-dihydroxycoumarin) (**1**), along with the known metabolites,* β*-sitosteryl stearate (**2**),* n*-tetracosanoic acid (**3**), friedelin (**4**), friedel-1-en-3-one (**5**),* β*-sitosterol (**6**), 29-norcycloartanol (**7**), oleanolic acid (**8**), 3-*O*-acetyloleanolic acid (**9**), 6-methoxy-7,8-methylenedioxy coumarin (**10**), and methyl-3-acetyloleanolate (**11**), were isolated from the roots of* Guazuma tomentosa*. The structures of these compounds were established on the basis of spectroscopic (IR, ^1H, ^{13}C NMR, and mass) studies.*

Keywords: *Guazuma tomentosa*, triterpenoids, coumarin, biscoumarin.

Guazuma tomentosa Kunth. syn. *G. ulmifolia* Lamk. (Sterculiaceae), a native of tropical America, belongs to a small genus of five species, out of which only one species is known in India. The fruit contains sweet edible mucilage, which causes diarrhea when eaten in excess. The roasted seeds are astringent and used in stomach trouble. The bark is tonic and demulcent. In the West India, the inner bark is used for the treatment of elephantiasis, and the infusion of old bark is considered sudorific and useful in cutaneous and chest diseases [1, 2]. Triterpenes, diterpenes, sesquiterpenes, flavonoids, and coumarins have been isolated from the stem bark [3, 4], leaves [5–7], heart wood [8, 9], and flowers [10], but any work on the chemistry of the roots has been lacking. Hence, we investigated the roots of this plant and herein report the isolation and structure elucidation of a new biscoumarin, 3,3′-methylenebis(4,6-dihydroxycoumarin) (**1**) from the combined chloroform-ethyl acetate fractions of the roots. In addition to this, *β*-sitosteryl stearate (**2**) [11], *n*-tetracosanoic acid (**3**) [12], friedelin (**4**) [13], friedel-1-en-3-one (**5**) [14], *β*-sitosterol (**6**) [11], 29-norcycloartanol (**7**) [13], oleanolic acid (**8**) [13], 3-*O*-acetyloleanolic acid (**9**) [13], 6-methoxy-7,8-methylenedioxycoumarin (**10**) [15], and methyl-3-acetyloleanolate (**11**) [13] were also isolated.

Compound **1** was obtained as light green needles, mp 241–243°C. In the UV spectrum, absorption at λ_{max} 330, 259, and 213 nm and blue-green fluorescence in UV light indicated its coumarin nature [16]. The mass spectrum afforded the molecular ion peak at *m/z* 368, consistent with the molecular formula of $C_{19}H_{12}O_8$. Its IR spectrum (KBr, cm^{-1}) shows absorptions at 3500–3300 (O-H), 1720, 1130 (coumarin lactone), and 1615, 1530, 1560 (aromatic C=C). The PMR and ^{13}C NMR spectra are given in Table 1. In the ^1H NMR spectrum, only three peaks were observed in the aromatic region: two doublets at δ 7.33 (J = 2 Hz) and δ 6.77 (J = 8 Hz) and one double of doublets at δ 7.27 (J = 8, 2 Hz). According to these data there were two possibilities corresponding to these aromatic protons: one is H-5, H-8, and H-6 and the other is H-5, H-8, and H-7. However, the most downfield doublet corresponding to H-5 appearing at δ 7.33 is meta-coupled, which confirmed the presence of protons at C-5, C-7, and C-8 and the hydroxy group at C-6. In addition to these, the singlet appearing at δ 3.40 could be assigned to the methylene (CH_2) joining the two 4,6-dihydroxycoumarin moieties at 3 and 3′ position.

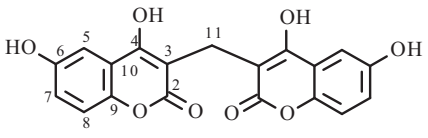

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TABLE 1. ^{1}H NMR and ^{13}C NMR Spectra of Compound **1**, (δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
2		168.2	7	7.27 (dd, J = 8, 2)	134.6
3		104.5	8	6.77 (d, J = 8)	126.7
4		172.4	9		154.7
5	7.33 (d, J = 2)	132.8	10		124.9
6		170.2	11	3.40 (s)	19.3

EXPERIMENTAL

General Experimental Procedures. Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus. IR spectra were recorded as KBr wafer phase with a system 8400 S model FTIR spectrophotometer (Schimadzu). NMR spectra were measured on model Jeol FX 90Q and Bruker DRX FT NMR at 200 MHz, using $CDCl_3$ and DMSO-d_6 as solvents and TMS as an internal standard. EI-MS spectra were taken on a Hitachi model RMU 6E and Jeol D-300 mass spectrometer. C and H analysis of the compounds was done on Coleman and Carlo Erba 1108 C and H analyzers.

Plant Material. The roots of *G. tomentosa* Kunth. were collected from the Rajasthan University campus, Jaipur and authenticated at the Herbarium, Department of Botany, University of Rajasthan, Jaipur (RUBL 19762), where a voucher specimen is deposited.

Extraction and Isolation. Air-dried and finely powdered roots (4.0 kg) were exhaustively extracted with ethanol (95%) on a steam bath for 8 h thrice, and the solvent was evaporated in vacuo. The extract (124 gm) was re-extracted with petroleum ether, benzene, chloroform, and ethyl acetate successively, which afforded the yellowish-brown petroleum ether (4.03 g), brown benzene (15.45 g), dark brown chloroform (4.76 g), and dark brown ethyl acetate (1.37 g) fractions upon drying under reduced pressure. TLC of these fractions in benzene–ethyl acetate (1:1) revealed a similarity between the petroleum ether and benzene fractions and the chloroform and ethyl acetate fractions. Similar fractions were pooled together and chromatographed over a column of silica gel using gradient elution with solvents of increasing polarity, viz. petroleum ether, benzene, ethyl acetate, and methanol where the petroleum ether–benzene fractions afforded compounds **1–10**, and the chloroform–ethyl acetate fractions afforded compound **11**.

3,3′-Methylenebis(4,6-dihydroxycoumarin) (1). Light green needles (51 mg); mp 241–243°C; ^{1}H and ^{13}C NMR data are given in Table 1; IR (KBr, ν, cm^{-1}): 3500–3200, 1720, 1615, 1570, 1560, 1130; MS: *m/z* 368 [M^+], 191 [$M^+ - C_9H_5O_4$], 177 [$M^+ - C_{10}H_7O_4$], etc.

***β*-Sitosteryl Stearate (2)**. $C_{47}H_{84}O_2$, colorless needles (106 mg), mp 84–85°C, $[\alpha]_D$ –26°.

***n*-Tetracosanoic Acid (3)**. $C_{24}H_{48}O_2$, colorless granules (77 mg), mp 84–86°C.

Friedelin (4). $C_{30}H_{50}O$, colorless needles (165 mg), mp 267–269°C, $[\alpha]_D$ –23°.

Friedel-1-en-3-one (5). $C_{30}H_{48}O$, colorless needles (95 mg), mp 228–229°C, $[\alpha]_D$ –73.8°C; IR (KBr, ν, cm^{-1}): 1680, 1620; MS: *m/z* 424 [M^+], 300 [$C_{21}H_{32}O]^+$, 271 [$C_{19}H_{27}O]^+$, 218 [$C_{16}H_{26}$, base peak]$^+$, 205 [$C_{15}H_{25}]^+$, 203 [$C_{15}H_{23}]^+$.

***β*-Sitosterol (6)**. $C_{29}H_{50}O$, white crystals (562 mg), mp 136–137°C, $[\alpha]_D$ –35°.

29-Norcycloartanol (7). $C_{29}H_{50}O$, white needles (98 mg), mp 131–133°C, $[\alpha]_D$ –42°C; IR (KBr, ν, cm^{-1}): 3350, 1120; ^{1}H NMR ($CDCl_3$, DMSO-d_6, δ, ppm): 3.46 (1H, m), 1.60–0.86 (47H, m), 0.45 (1H, d), 0.20 (1H, d); MS: *m/z* 414 [M^+].

Oleanolic Acid (8). $C_{30}H_{48}O_3$, colorless needles (199 mg), mp 304–305°C.

3-*O*-Acetyloleanolic Acid (9). $C_{32}H_{50}O_4$, white needles (931 mg), mp 255–257°C; IR (KBr, ν, cm^{-1}): 3300–2550, 1740, 1700, 1260; ^{1}H NMR ($CDCl_3$, DMSO-d_6, δ, ppm): 5.25 (1H, d), 4.50 (1H, t), 2.05 (3H, s), 1.85–0.7 (44H, m); MS: *m/z* 498 [M^+], 248 (base peak).

6-Methoxy-7,8-methylenedioxy Coumarin (10). $C_{11}H_8O_5$, colorless needles (189 mg), mp 219–221°C; IR (KBr, ν, cm^{-1}): 1720, 1620, 1590, 1560, 1130; ^{1}H NMR ($CDCl_3$, DMSO-d_6, δ, ppm, J/Hz): 7.27 (1H, d, J = 9.6, H-3), 6.27 (1H, d, J = 9.6, H-4), 3.93 (1H, s, H-5), 6.16 (2H, s, -O-CH_2-O-); MS: *m/z* 220 [M^+, base peak], 205 [M^+ – 15], 192 [M^+ – 28], 177 [M^+ – 43].

Methyl-3-acetyloleanolate (11). $C_{33}H_{52}O_4$, white needles (101 mg), mp 216–219°C, $[\alpha]_D$ –79°C; IR (KBr, ν, cm^{-1}): 1750, 1390, 1360, 1325, 1260, 1250; MS: *m/z* 512 [M^+], 497 [$M^+ - CH_3$], 452 [$M^+ - CH_3COOH$], 437 [M^+ – 75], 203 [base peak].

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