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A BENZOIC ACID ESTER FROM *UVARIA NARUM*

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Key Word Index—*Uvaria narum*; *U. hookeri*; Annonaceae; glut-5(6)-en-3-ol; betulapren-11-ol; 2-*E*-[2″-oxo-cyclopent-3″-en-1″-ylidene]ethyl benzoate; X-ray crystal structure.

Abstract—A new benzoic acid ester, 2-*E*-[2″-oxo-cyclopent-3″-en-1″-ylidene]ethyl benzoate, together with tritriacontane, tetratriacontanol and β-sitosterol have been isolated from a petrol extract of the leaves of *Uvaria narum*. The structure of the new ester has been established on the basis of spectral data and finally confirmed by X-ray crystallography. Glut-5(6)-en-3-ol and betulapren-11-ol have also been isolated from a petrol extract of the leaves and stems of *U. hookeri*; this is the first report of the isolation of the latter compound from the genus *Uvaria*.

INTRODUCTION

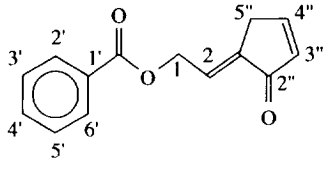
Uvaria species have been used as medicines and tonics in many parts of the world. Different parts of *Uvaria* species have been used as preservatives, fragrants and medicinal agents in jaundice, biliousness, abdominal pains and fever. Many compounds having physiological activity have been reported earlier from this genus [1–4]. As part of our research programme on the isolation of physiologically active compounds [5, 6], we undertook the phytochemical investigations of *U. narum* and *U. hookeri*. In all, six compounds were isolated from the petrol extracts of the leaves, and leaves and stems of *U. narum* and *U. hookeri*, respectively.

RESULTS AND DISCUSSION

Column chromatography of the concentrated petrol extract of the air-dried leaves of *U. narum* led to the isolation of four compounds, a novel benzoic acid ester (**1**), tritriacontane, tritriacontanol and β-sitosterol.

Compound **1** was isolated as a crystalline solid from petrol–chloroform (3:2), mp 78°. Its molecular formula, $C_{14}H_{12}O_3$ was determined by mass spectrometry ($[M]^+$ *m/z* 228) in combination with hydrogen and carbon counts from NMR spectra and further confirmed by its FAB-mass spectrum (2 $[M]^+ + 1$ at *m/z* 457). In its ^{1}H NMR spectrum, peaks at δ8.05 (2H), 7.58 (1H) and 7.46 (2H) indicated the presence of a monosubstituted benzene ring containing an electron-withdrawing group, which was indicated to be a carbonyl (C=O) by the presence of a peak at *m/z* 105 $[C_6H_5CO]^+$ in the mass spectrum. The peaks at δ166.2 and 62.1 in the ^{13}C NMR spectrum gave evidence for the presence of an ester

1



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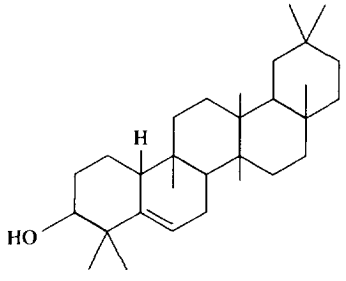

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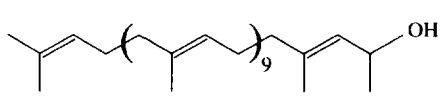


linkage in the molecule. The peak at *m/z* 123 $[M - C_6H_5CO]^+$ together with the base peak at *m/z* 105 in its mass spectrum gave confirmation of the presence of a benzoate moiety in the molecule. The presence of peaks at δ6.42 (1H) and 7.64 (1H) in the ^{1}H NMR spectrum of **1**, together with a peak at δ196.0 in its ^{13}C NMR spectrum,

suggested the presence of an α,β-unsaturated carbonyl group. The presence of two carbonyl groups in the molecule was also indicated by peaks at 1720 (ester CO) and 1698 cm^{-1} (α,β-unsaturated CO) in the IR spectrum. Well-resolved multiplets at δ6.72 (1H), 5.05 (2H) and 3.35 (2H) were indicative of the presence of two allylic methylene groups in the molecule. Careful analysis of the information provided by the ^1H, ^{13}C NMR and mass spectral data reveals the presence of a cyclopentenone ring system with an exocyclic double bond. Thus, on the basis of the above spectral data, we propose the structure of **1** to be 2-*E*-[2″-oxo-cyclopent-3″-en-1″-ylidene]ethyl benzoate. Complete assignments of the various signals are shown in Table 1 together with multiplicities and correlations obtained from a ^1H–^1H COSY experiment. The *trans*-geometry of the exocyclic double bond is in accordance with the observed NOE on H-1 upon irradiation of H-5″ and vice versa. Also, no enhancement was observed on the H-2 signal on irradiation of H-5″ and vice versa (Table 2). The proton–carbon connectivities in structure **1** were confirmed by an HMQC experiment. In order to confirm the structure, a single crystal X-ray diffraction analysis of **1** was undertaken. The X-ray molecular model of the ester (Fig. 1) confirms all the above deductions related to the structure of compound **1** and established the geometry of the exocyclic double bond. Atomic coordinates and selected bond lengths and bond angles are deposited at the Cambridge Crystallographic Data Centre, U.K.

Benzoic acid esters occur commonly in nature and numerous compounds of this type have been isolated from different plant species. However, this is the first report of a compound from a natural source having an unusual alcohol moiety [2-*E*-(2-oxo-cyclopent-3-en-1-ylidene)ethyl alcohol], based on a cyclopentenone ring. The genus *Uvaria* is a source of a number of novel compounds [7]; cyclohexene oxides belong to one such class of compounds for which a biogenetic route has been

Table 1. ^1H (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) spectral data of **1** with respect to TMS

Site	^{13}C δ[ppm]	^1H δ[ppm]	m	I(^1H)	J_{HH}[Hz]	Couples to
1″	129.6	—	—	—	—	—
2″	196.0	—	—	—	—	—
3″	135.8	6.42	*dt*	1	5.99	**4″**
					2.23	5″
4″	157.6	7.64	*dtd*	1	5.96	**3″**
					2.60	5″
					1.02	(2)
5″	32.1	3.35	*m*	2	—	2, 1, 3″, 4″
COO	166.2	—	—	—	—	
1′	135.8	—		—		
2′, 6′	129.7	8.05	*m*	2	—	**3′**, 4′, 5′
3′, 5′	128.5	7.46	*m*	2	—	**2′**, **4′**, **6′**
4′	133.3	7.58	*m*	1	—	**2′**, **3′**, **5′**, **6′**
1	62.1	5.05	*dt*	2	6.02	**2**
					0.91	5″
2	127.7	6.72	*ttd*	1	6.02	**1**
					1.86	5″
					1.03	(4″)

One-bond connectivities are given in bold. Probable connectivities in parentheses do not show in the DQ-COSY, but follow from the analysis of the ^1H spectra. Proton–carbon connectivities were obtained from HMQC.

Table 2. NOE results of **1**

Irradiation	Enhancements (%) H-3″	H-4″	H-5″	H-2′, 6′	H-1	H-2
H-3″	—	1.8	—	—	—	—
H-4″	1.8	—	1.6	—	—	—
H-5″	—	7.5	—	—	1.32	—
H-3′, 4′, 5′	—	—	—	16.3	—	—
H-1	—	—	1.1	—	—	5.0
H-2	—	—	—	—	0.75	—

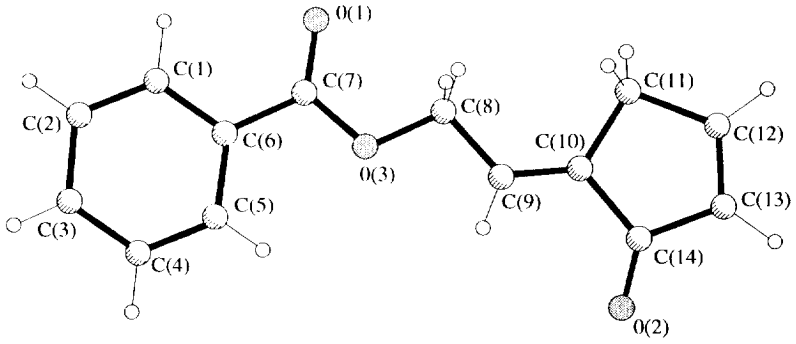
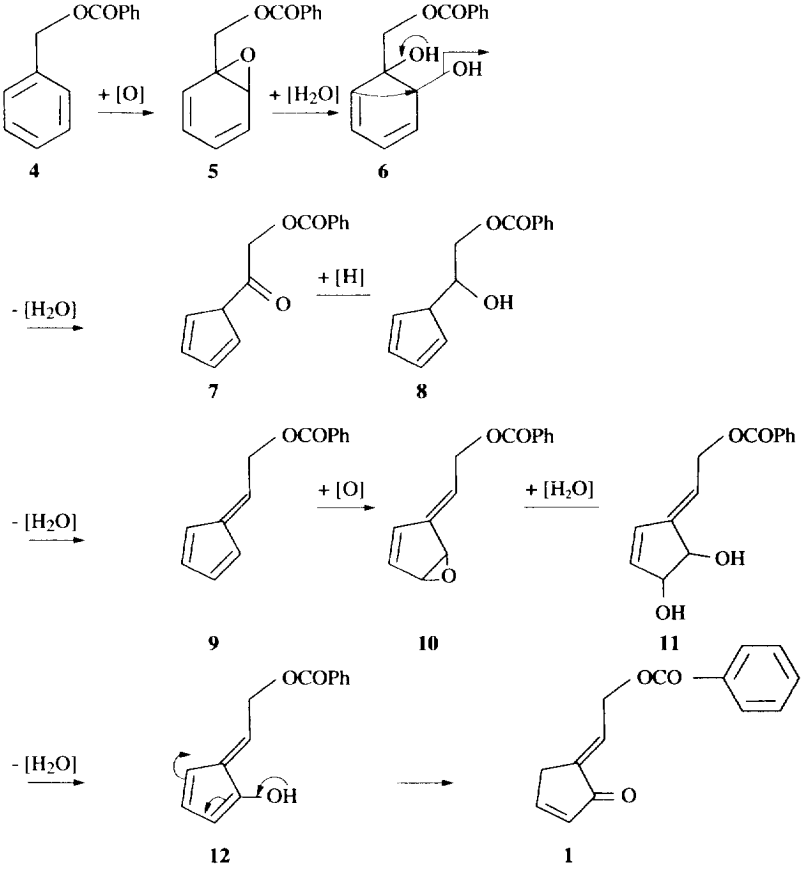


Fig. 1. Molecular structure of **1**.



Scheme 1. Possible steps in the biosynthesis of **1** from benzyl benzoate (**4**).

mapped [8]. On similar lines, we believe the compound **1** finds its origin from benzyl benzoate (**4**), which is a common constituent of most of the *Uvaria* species [6], including *U. narum* [9]. We propose that benzyl benzoate (**4**) is epoxidized to the key intermediate **5**, the epoxide ring then opens up and undergoes a ring contraction followed by reduction and water elimination to give **9** through the formation of intermediates **6**–**8** (Scheme 1). Compound **9** then undergoes epoxidation to **10**, followed by epoxide ring opening to give **11**, which in turn undergoes elimination of water to give **12**. The enol **12** tautomerizes to its keto form **1**, i.e. the title compound.

Tritriacontane [10] was isolated earlier from *Calocephalus brownii* F. Muell. by Batterham *et al.* [11] as a mixture with two other long-chain hydrocarbons *n*-$C_{29}H_{60}$ (37%) and *n*-$C_{31}H_{64}$ (51%). Thus, this is the first report of its isolation from a natural source in a pure form. Tetratriacontanol was identified on the basis of its spectral data (^1H, ^{13}C NMR and EI-mass spectrum) and comparison of its mp with literature values [12].

In addition, compounds **2** and **3** were isolated from a concentrated petrol extract of the leaves and the stems of *U. hookeri*. Compound **2** was isolated as needles. On the basis of its spectral analysis (IR, UV, ^{1}H and ^{13}C NMR and EI-mass spectrum) and comparison of its mp with the literature values, it was found to be glut-5(6)-en-3-ol, isolated earlier from *Euphorbia cyparissias* [13–15]. Compound **3** was characterized as betulapren-11-ol by comparison of its spectral data with those in the literature [16, 17]. This is the first report of the isolation of the C_{55}-isoprenoid alcohol **3** from the genus *Uvaria*.

EXPERIMENTAL

Mps: uncorr. IR: KBr, Nujol. UV: MeOH. ^{1}H NMR: 250 or 500 MHz. ^{13}C NMR: 62.9 or 125 MHz. EIMS or FAB-MS techniques were used for MS analysis. Silica gel (60–80 mesh) was used for CC and silica gel G was used for TLC and prep. TLC.

Plant material. Leaves and stems of *U. narum* and *U. hookeri* were collected from the campus of the National Research Centre for Spices, Calicut, Kerala (India) in November 1990.

Extraction and isolation. Air-dried leaves of *U. narum*, and leaves and stems of *U. hookeri* were extracted separately and successively with petrol (60–80°), CH_2Cl_2 and MeOH in a Soxhlet apparatus. The extracts were concd *in vacuo*. The petrol extract of the leaves of *U. narum* was subjected to CC and eluted with petrol and increasing concns of $CHCl_3$ in petrol. Compound **1** and β-sitosterol crystallized out from frs eluted with 10% $CHCl_3$ in petrol and 25% $CHCl_3$ in petrol, respectively. Tritriacontane and tetratriacontanol were purified from the fr. eluted with 1% $CHCl_3$ in petrol by prep. TLC. Similarly, the petrol extract of leaves and stems of *U. hookeri* was subjected to CC and eluted with petrol and increasing concns of $CHCl_3$ in petrol. Compounds **2** and **3** were purified from a fr. eluted with 15% $CHCl_3$ in petrol by prep. TLC.

2-E-[2″-*Oxo-cyclopent*-3″-*ene*-1″-*ylidene*]*ethyl benzoate* (**1**). Recrystallized from petrol–EtOAc as crystals (200 mg), mp 78°. IR ν_{max}^{KBr} cm^{-1}: 1720, 1698, 1657, 1269. UV λ_{max}^{MeOH} nm: 245. ^{1}H NMR ($CDCl_3$): δ3.35 (2H, *m*, H-5″), 5.05 (2H, *dt*, *J* = 6.0, 0.9 Hz, H-1), 6.42 (1H, *dt*, *J* = 6.0, 2.2 Hz, H-3″), 6.72 (1H, *ttd*, *J* = 6.0, 1.9, 1.0 Hz, H-2), 7.46 (2H, *m*, H-3′ and H-5′), 7.58 (1H, *m*, H-4′), 7.64 (1H, *dtd*, *J* = 6.0, 2.6, 1.9 Hz, H-4″), 8.05 (2H, *m*, H-2′ and H-6′). ^{13}C NMR ($CDCl_3$): δ32.1 (C-5″), 62.1 (C-1), 127.7 (C-2), 128.5 (C-3′ and C-5′), 129.6 (C-1″), 129.7 (C-2′ and C-6′), 133.3 (C-4′), 135.8 (C-1′), 135.8 (C-3″), 157.6 (C-4″), 166.2 (COO), 196.0 (C-2″). EIMS *m/z* (rel. int.): 228 $[M]^+$ (3.0), 123 $[M - C_6H_5CO]^+$ (1), 105 $[C_6H_5CO]^+$ (100), 77 $[C_6H_5]^+$ (44), 51 (16), 39 (8). FAB-MS: 457 $[2M + 1]^+$ (2).

Tritriacontane. Recrystallized from hexane as a solid (150 mg), mp 70–71° (lit. [10] mp 71.8°). IR ν_{max}^{Nujol} cm^{-1}: 2950, 1450, 1370. ^{1}H NMR ($CDCl_3$): δ0.90 (6H, *t*, *J* = 7 Hz, 2× – Me), 1.30 (62H, *br s*, Me (CH_2)$_{31}$ Me). ^{13}C NMR ($CDCl_3$): δ14.0 (C-1 and C-33), 23.0 (C-2 and C-32), 29.5 (C-4 to C-30), 31.8 (C-3 and C-31). EIMS *m/z* (rel. int.): 464 $[M]^+$ (1), 436 (3), 408 (2), 127 (4), 113 (6), 99 (10), 85 (40), 71 (58), 57 (100), 43 (65), 29 (5).

Tetratriacontanol. Powder (200 mg), mp 86–87° (lit. [12] mp 92°). IR ν_{max}^{Nujol} cm^{-1}: 3300, 1450, 1375. ^{1}H NMR ($CDCl_3$): δ0.90 (3H, *t*, *J* = 7 Hz, Me), 1.30 (62H, *br s*, Me (CH_2)$_{31}$$CH_2$), 1.55 (2H, *m*, –$CH_2$–$CH_2OH$), 3.65 (2H, *t*, *J* = 7 Hz, CH_2–OH); ^{13}C NMR ($CDCl_3$): δ13.9 (C-34), 22.5 (C-33), 29.6 (C-3 to C-32), 32.8 (C-2), 63.03 (C-1); EIMS *m/z* (rel. int.): 476 $[M - 18]^+$ (6), 448 (14), 420 (20), 392 (12), 125 (17), 111 (34), 97 (63), 83 (78), 69 (62), 57 (100), 43 (70).

Glut-5(6)-en-3-ol (**2**). Recrystallized from $CHCl_3$–petrol (4:1) as needles (150 mg), mp 208–210° (lit. [15] mp 206–208°). IR ν_{max}^{KBr} cm^{-1}: 3475, 2975, 1600, 1480, 1350, 1350. UV λ_{max}^{MeOH} nm: 225.6. ^{1}H NMR ($CDCl_3$): δ0.85, 0.95, 0.99, 1.01, 1.04, 1.09, 1.14 and 1.16 (24H, all singlets, 8 × Me), 1.24–1.90 (23H, *m*, 10 × CH_2 and 3 × CH), 3.46 (1H, *m*, –CH–OH), 5.66 (1H, *m*, =CH). ^{13}C NMR ($CDCl_3$): δ16.2, 18.2, 18.4, 19.6, 23.6, 25.4, 27.8, 28.2, 28.8, 30.1, 30.3, 33.0, 33.1, 34.5, 34.6, 34.8, 35.1, 36.0, 37.8, 38.1, 38.3, 40.8, 43.1, 47.4, 48.9, 76.3, 122.1, 141.6. EIMS *m/z* (rel. int.): 426 $[M]^+$ (28), 408 $[M - H_2O]^+$ (7), 274 (100), 259 (63), 205 (22), 134 (25), 95 (15), 81 (9), 69 (7).

Betulapren-11-ol (**3**). Purification by prep. TLC yielded a viscous oil (50 mg) identified by comparison of its IR, ^{1}H NMR and EI-MS data with those reported in refs [16, 17].

X-ray crystallography of ester **1**. Crystal data: $C_{14}H_{12}O_3$, $M = 228.24$, triclinic, P_1; $a = 6.018$ (4), $b = 7.593$ (2), $c = 13.066$ (3)Å, $\alpha = 98.44$ (2), $\beta = 91.98$ (4), $\gamma = 102.46$ (4)°, $U = 575.3$ (4)Å^3, $Z = 2$, $T = 293$ (2) K; refined [18, 19] by full-matrix least-squares on F^2 for 1476 unique reflections; $\omega R2 \sim 0.1559$ for all data and $R_1 = 0.051$ for 1267 reflections with $I > 2\sigma(I)$. All measurements were made using a Siemens P3R3 four-circle diffractometer equipped with an Oxford Cryosystems Cryostream Cooler (version 2.4). Graphite monochromated Mo-Kα radiation ($\lambda = 0.71073$ Å) was used to collect the intensity data in the w-20 mode. Unit-cell parameters and orientation matrices were obtained by least-squares refinement of the setting angles of 20 high-angle reflections. The crystallographic program system was SHELXTL PLUS [18] and SHELXL-93 [19]; the refinement program uses atomic scattering factors taken from International Tables for Crystallography [20]. The structures were solved by direct methods and refined using full matrix least-squares on F^2 (for 2) or F (for 1). All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were inserted using a riding model and given isotropic thermal parameters equal to 1.2- (or 1.5- for methyl groups) times the equivalent isotropic displacement parameter of the atom to which it is attached (compound **1** was given a fixed isotropic displacement parameter of 0.08). The weighting scheme was of the form $\omega = 1/(\sigma^2(F) + gF^2)$ (for 1) or $\omega^{-1} = [\sigma^2(F)^2 + (aP)^2 + bP]$ where $P = [\text{max.}\ (F_0^2, 0) + 2F_c^2\ 1]/3$ (for 2). The R factors are defined as $R(F) = \Sigma \| F_o | - | F_c \| / \Sigma | F |$ and $\omega R(F^2) = [\Sigma[\omega(F_0^2 - F_c^2)^2]/\Sigma[\omega(F_c^2)^2]]^4$. The complete data are deposited at the Cambridge Crystallographic

Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

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