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CHEMISTRY OF THE ANNONACEAE, PART XXVI. THE UVARISESQUITERPENES, A NOVEL TYPE OF BENZYLATED SESQUITERPENE FROM *UVARIA ANGOLENSIS*

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ABSTRACT.—Three novel compounds in which a sesquiterpene nucleus is coupled with an *o*-hydroxybenzyl unit and where cyclization has occurred through the phenolic hydroxyl to give a benzopyran have been isolated from the stem bark of *Uvaria angolensis*. These sesquiterpenes, which have been given the trivial names uvarisesquiterpenes A–C, are based on germacrane (uvarisesquiterpene A), selinane (uvarisesquiterpene B), and axane (uvarisesquiterpene C) skeletons.

Uvaria L. (Annonaceae) is a genus of straggling or climbing shrubs or, rarely, trees found throughout the tropics and subtropics of the Old World (1). Chemical investigations of several African species have revealed the presence of a number of dihydrochalcones and flavanones substituted with *o*-hydroxybenzyl or methyl units (2, 3) and derivatives of syncarpic acid (4). Other species of both African (5) and Asian (2, 6–8) origin have yielded polyoxygenated cyclohexane derivatives incorporating benzoyl esters. Thus, it appears that a general theme running through the chemistry of *Uvaria* is the ability to employ benzyl or benzoyl groups to substitute a number of different types of secondary metabolite.

Uvaria angolensis Oliv. is found throughout tropical Africa and was one of the first species to be examined chemically, root bark collected in Nigeria yielding eight *C*-benzylated or *C*-methylated dihydrochalcones and flavanones (3). In East Africa *U. angolensis* occurs, as the distinct variant A, in Western Tanzania and Zambia (9). We have examined the stem bark of this variant collected in the Gombe National Park, Tanzania, and in previous papers we have reported the presence of benzyl benzoate, a number of known *C*-benzylated dihydrochalcones (10), and four unique *C*- and *N*-benzylated indoles, uvarindoles A–D (10, 11). In this paper we wish to report the occurrence of yet another type of *C*-benzylated metabolite from *Uvaria*, benzylated sesquiterpenes.

RESULTS AND DISCUSSION

Using cc followed by circular preparative tlc, the petroleum ether extract of the stem bark yielded three amorphous solids each of which appeared pure by tlc and analyzed for 22 carbons. The major of these (yield 0.07%), $C_{22}H_{32}O_2$ by accurate mass measurement eims, gave a uv spectrum for a simple aromatic compound. The ^{1}H-nmr spectrum (Table 1) revealed four aromatic protons as an ABCD system, two olefinic protons, and signals for four methyl groups. The mass spectrum gave a base peak *m/z* 107 $[C_7H_7O]^+$ which, together with nmr and uv spectra, was indicative of the *o*-hydroxybenzyl moiety (10). Accepting the presence of this unit leaves $C_{15}H_{26}O$, a sesquiterpene.

The ^{13}C-nmr spectrum (Table 2) gave, in addition to the anticipated resonances for the hydroxybenzyl, two singlets, at 75.35 and 80.44 ppm, for oxygen-bearing quaternary carbons and doublets at 134.30 and 136.33 ppm for an olefinic bond. On acetylation a monoacetate was obtained, and this caused no change in the resonance positions

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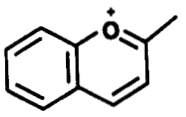
TABLE 1. ^{1}H-nmr Chemical Shift Values and Coupling Constants (in parentheses).[a]

Proton	Compound				
	4	Acetate of **4**	Epoxide of **4**	**6**	**9**
H-1	1.24–1.28 1.84–1.87	1.18–1.22 2.09–2.13	—	—	1.24–1.26
H-2	0.99–1.04 1.41–1.47	0.97–1.03 1.54–1.65	—	—	1.59–1.80
H-3	1.75–1.81	1.74–1.78	—	—	1.47
H-5	5.49 d (16.5)	5.57 d (16.4)	2.84 br s	—	1.39 d (11.3)
H-6	5.41 dd (16.5, 6.4)	5.44 dd (16.4, 7.0)	2.80 dd (9, 2)	—	2.15 m
H-7	1.97–2.02	2.01–2.05	—	—	3.37 dd (9.6, 1.8)
H-8	1.84–1.96	1.54–1.65 1.82–1.96	—	5.33 dd (5.3, 2.3)	1.40–1.80
H-9	1.53–1.63 1.75–1.81	1.74–1.80	—	2.18–2.20	1.42–1.78
H-11	1.53–1.63	1.74–1.80	—	1.90–2.00	1.90
Me-12 Me-13	0.91 d 0.94 d (6.6)	0.92 d 0.93 d (6.6)	1.00 d 1.05 d (6)	1.02 d 1.03 d (6.8)	0.88 d 0.99 d (6.9)
Me-14	1.36 s	1.58 s	1.08[b] s	0.81 s	0.87 s
Me-15	1.06 s	1.05 s	1.02[b] s	1.22 s	1.26 s
H-α_{ax}	2.39 dd (16.4, 12.1)	2.39 dd (16.4, 12.2)	2.45 dd (15, 11)	2.07 dd (13.1, 11.2)	2.15 dd (13.1, 10.6)
H-α_{eq}	2.85 dd (16.4, 5.1)	2.85 dd (16.4, 5.1)	2.96 dd (15, 5)	2.89 dd (13.1, 2.0)	2.81 dd (13.1, 2.5)
H-3′	6.73 dd (8.1, 1.0)	6.72 dd (8.1, 1.0)	6,75 d (7.5)	6.71 dd (7.5, 1.0)	6.72 dd (7.6, 1.5)
H-4′	7.06 t (7.4)	7.05 t (7.3)	7.05 t (7.5)	7.04 td (7.5, 1.0)	7.01 td (7.6, 1.0)
H-5′	6.78 ddd (8.1, 7.4, 1.0)	6.77 ddd (8.1, 7.3, 1.0)	6.79 td (7.5, 1)	6.80 td (7.5, 1.0)	6.75 td (7.6, 1.5)
H-6′	7.01 d (7.4)	6.99 d (7.3)	7.09 d (7.5)	7.02 dd (7.1, 1.0)	6.97 dd (7.6, 1.5)
Ac	—	1.98 s	—	—	—

[a]All spectra run at 360 MHz except for the epoxide of **4** (90 MHz).
[b]May be interchanged.

of the aromatic protons. This, together with the absence of a bathochromic shift in the uv spectrum on addition of NaOH and the presence of a significant ion at *m/z* 145 which is attributable to the methylbenzopyran nucleus [**1**], indicated that the hydroxybenzyl system had cyclized in the manner noted previously in the dihydrochalcone chamuvaritin (2). The presence of the benzopyran nucleus was further supported by the ^{1}H-nmr spectrum, which showed the AB portion of an ABX system centered at δ 2.39 and 2.85 for the benzyl (α) protons. These showed coupling to a single, axial, ring junction proton ($J_{ax/ax}$ 12.1 Hz). Through a series of decoupling and COSY experiments it was possible to expand the benzopyran skeleton to the partial structure **2**.

Given partial structure **2** and the presence of a disubstituted olefinic bond, only a monocyclic sesquiterpene was possible. Further decoupling and COSY ^{1}H-nmr analyses showed the unresolved portion of the structure to contain an isopropyl unit and a *trans*-substituted double bond, one carbon of which was linked to a quaternary center



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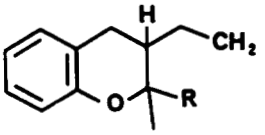

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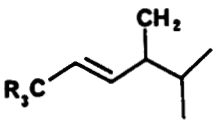

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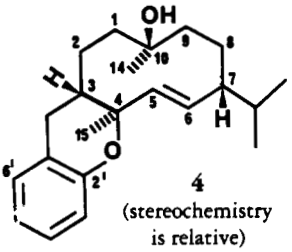

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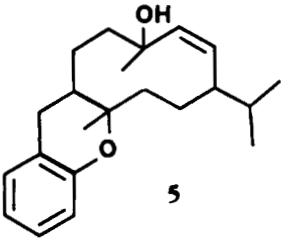

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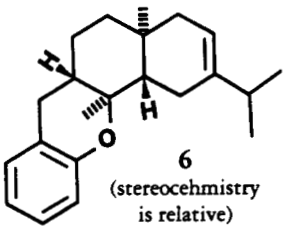

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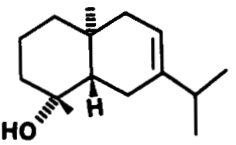

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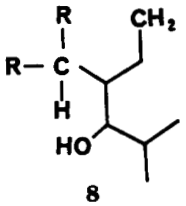

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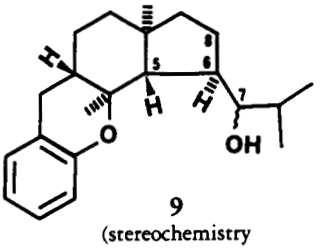

9
(stereochemistry is relative)

and the other to the same methine as the isopropyl unit. This system could be further expanded by the addition of a methylene to give a second partial structure, **3**. Structures **2** and **3** incorporate all except one methylene and a quaternary carbon, which must be substituted with both a methyl and an hydroxyl. Linking **2** and **3**, with incorporation of the additional carbons, gives two possible structures, **4** and **5**, based on a germacrane nucleus.

These structures were tested by a series of nOe studies. Irradiation of either olefinic proton caused an enhancement of the methyl signal at δ 1.36. This signal must be assigned to the C-10 methyl because of the large (0.22 ppm) shift that occurs on acetylation. The nOe suggests that this methyl must lie opposite the olefinic center across the 10-membered germacrane ring. This was substantiated by reversal of the procedure; irradiation at δ 1.36 caused enhancement of both H-5 and H-6 (not possible in **5**) and, in addition, of the other (C-4) methyl singlet at δ 1.06. Lastly, irradiation of the C-4 methyl led to enhancement of the C-10 methyl and, in addition, of the axial H-α and an

TABLE 2. ^{13}C-nmr Chemical Shift for Benzylated Sesquiterpenes.[a]

Carbon	Compound				
	4	Acetate of 4	6	7(13)	9
C-1	40.34*	37.48	42.69**	41.3	39.06*
C-2	29.03*	29.00*	23.15**	18.4	24.10**
C-3	36.68	36.65	49.41**	41.6	49.08***
C-4	80.44	80.27	72.95	71.6	72.45
C-5	136.33	133.91**	51.17***	47.3	63.26
C-6	134.30	133.65**	30.38	30.2	46.83***
C-7	45.38	45.34	141.75	142.5	79.96
C-8	31.15*	31.34*	116.11	116.6	32.46
C-9	27.99*	26.47*	42.99	44.6	42.36*
C-10	75.35	85.04	36.40	32.5	41.84
C-11	30.58	30.73	34.86	35.1	30.74
C-12	21.21	21.25	21.16	21.3	16.22
C-13	20.54	20.47	21.67	21.9	20.58
C-14	25.40	21.80	13.58	18.0	13.84
C-15	20.97	20.93	22.78	23.3	23.41
C-α	26.69*	26.71*	24.87**		25.68**
C-1′	121.25	121.14	127.84		127.73
C-2′	153.77	153.78	153.82		154.16
C-3′	116.39	116.46	115.14		115.12
C-4′	127.12	127.16	126.90		126.96
C-5′	119.11	119.33	120.16		119.73
C-6′	128.64	128.64	131.36		131.14

[a]Assignments in the same column with the same number of asterisks are interchangeable.

H-1 proton. On this basis structure **4** is correct with the two methyl groups at C-10 and C-4 on the same side of the ring as the axial H-α. This requires that the H-3 proton is axial and on the other face (here depicted as β).

Epoxidation of **4** proceeded with ease and gave a single product in which the ^{1}H-nmr resonance for the C-10 methyl underwent a marked shielding to δ 1.08. The ^{13}C-nmr spectra of **4** and compound **4** acetate were assigned (Table 2) by comparison with published data for germacrane (12) and *o*-hydroxybenzyl compounds (10) and by a partial HETCOR study performed on **4**.

For the second compound (yield 0.01%) hreims indicated an empirical formula $C_{22}H_{30}O$. Examination of uv, ^{1}H-nmr, and eims spectra indicated the same benzopyran nucleus as **4** and four methyl groups (two doublets, two singlets), but only one olefinic proton which resonated as a double doublet. One methyl singlet was markedly shielded (δ 0.81). The ^{13}C-nmr spectrum (Table 2) confirmed the presence of a trisubstituted olefin. From these data this must be a bicyclic sesquiterpene with a benzopyran system comparable to that of **4** and with one center of unsaturation. (In this paper numbering of all sesquiterpenes is based in the germacrane nucleus so that nmr arguments and tables are simplified.)

Decoupling experiments performed on the methyl doublets showed them coupled to H-11 centered at δ 1.95. The relative deshielding of H-11 (δ 1.60 in **4**) and absence of further coupling require that the double bond be placed at C-7 with the olefinic proton at C-8, given that it shows two couplings, requiring structure **6** for the second sesquiterpene. The ^{13}C-nmr spectrum (Table 2) corresponds well with that reported (13) for 4-(*R*)-hydroxyselin-7-ene [**7**]. In **6** the highly shielded ^{13}C-nmr resonance for the C-10 methyl (13.58 ppm) and correspondingly deshielded resonance for C-5 (49.51 ppm) indicate a *trans* ring junction. In decalins with a *cis* ring junction the C-10 methyl is

strongly deshielded and C-5 resonates at ca. 42 ppm (13–16). The deshielding of C-2 in **6** (cf. in **7**; Table 2) is attributed to the C-3 benzyl substituent, the observed resonance being in good agreement with that published for C-2 of 1-methyldecalins (17).

The final isolate (0.003%), $C_{22}H_{32}O_2$ by accurate mass measurement, again showed spectral characteristics for a benzopyran nucleus. The second oxygen was present as a secondary alcohol (79.96 ppm, δ 3.37, dd). Decoupling experiments, commencing with the irradiation of two methyl doublets (Table 1), revealed the presence of the partial structure **8** with the isobutanol moiety being confirmed by the occurrence of major ions at *m/z* 73 $[C_4H_9O]^+$ and 55 $[73 - H_2O]^+$.

As both ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) indicated that the benzopyran nucleus and C-1 to C-4 were comparable to **6**, this minor compound must have the rare axane skeleton and structure **9**. The methine carbon at 63.26 ppm can be assigned to C-5, where it is comparable to the value of 60.7 ppm recorded for C-5 in the related axane oppositol (18). The proposed relative stereochemistry of **9** was supported by nOe as follows: (a) Irradiation of the C-4 methyl enhanced the C-10 methyl and the axial H-2 proton, requiring both methyls to be axial and on the same face; (b) Irradiation of the C-10 methyl enhanced H-6 as well as the C-4 methyl, requiring H-6 to be on the same face as the methyls.

The benzylated sesquiterpenes **4**, **6**,and **9** have been assigned the trivial names uvarisesquiterpenes A, B, and C, respectively. They appear to represent the first isolation of compounds in which the *o*-hydroxybenzyl group has linked with a sesquiterpene. Although three different sesquiterpene ring systems occur, these compounds are clearly related. Routes can be envisaged for the formation of **6** and **9** from **4** initiated by loss of the 10-hydroxyl function with formation of a carbocation. It is also possible to think of **6** and **9** as artifacts produced from **4** during extraction. Indeed, if **4** is subjected to prolonged exposure to Si gel, several products are obtained. These include both **6** and **9**, but in trace amounts only.

EXPERIMENTAL

Uv spectra were run in MeOH; ir spectra were obtained as KBr discs or liquid film; nmr spectra were run at 360 MHz (^{1}H) and 90.56 MHz (^{13}C) in $CDCl_3$ using TMS as internal standard. COSY ^{1}H spectra were obtained at 300 MHz using 90° pulse width and 2D spectral width of 2102.2 Hz. HETCOR spectra were obtained at ^{13}C frequency of 75.4 MHz with spectral width 10,977 Hz, pulse width 90°. Eims were run at 70 eV with probe temperature of 120°. Optical rotation measurements were made with a Perkin-Elmer 240 polarimeter.

PLANT MATERIAL.—Stem bark of *U. angolensis* was collected from the Gombe National Park, Tanzania, in 1979. A voucher specimen is deposited at the East African Herbarium, Nairobi, as part of the collection made in Gombe by B. Verdcourt.

EXTRACTION AND ISOLATION OF SESQUITERPENES.—The ground stem bark (500 g) was extracted with petroleum ether (b.p. 60–80). After concentration, the extract was chromatographed over a column of Si gel. Elution with toluene-EtOAc-HOAC (98:2:0.1) gave a mixture of simple sesquiterpenes. Further elution with the same solvent in the proportions 96:4:0.1 gave a mixture of compounds including **4** and **6**. This mixture was separated by circular preparative tlc on Si gel (solvent, as above, 98:2:0.1) to give **4** (350 mg) and **6** (50 mg). Further elution of the column with the same solvent in the proportions 93:7:0.1 gave another mixture containing **9**, which was again separated by preparative tlc (solvent ratio 90:10:0.1) to give **9** (15 mg).

UVARISESQUITERPENE A [**4**].—Amorphous, $[\alpha]_D$ −16° ($c = 0.12$, $CHCl_3$); uv λ max nm (log ε) 220 (4.10), 274 (3.96), 282 (3.95); ir ν max cm^{-1} 3400, 1595, 1495, 1450, 1260, 1100, 800, 750; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 328 (37), 310 (10), 295 (9), 267 (7), 221 (22), 203 (60), 159 (15), 145 (28), 123 (26), 107 (100); calcd for $C_{22}H_{32}O_2$, 328.3404; found (eims) 328.3408.

UVARISESQUITERPENE A ACETATE.—Compound **4** (50 mg) in pyridine (5 ml) was treated with

Ac_2O at 50° for 24 h. Normal work-up gave the acetate (35 mg) as a gum; ir ν max cm^{-1} 1740, 1595; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

UVARISESQUITERPENE A 5,6-EPOXIDE.—Compound **4** (80 mg) in CH_2Cl_2 (20 ml) was treated by dropwise addition of *m*-chloroperoxybenzoic acid at room temperature. A 5% solution of sodium metabisulfate (10 ml) was added, and the mixture was stirred for 25 min. The reaction mixture was treated with 10% $NaHCO_3$, and the epoxide was obtained from the organic phase as an oil: $[\alpha]_D$ −21° (c = 0.1, $CHCl_3$); ^1H nmr see Table 1.

UVARISESQUITERPENE B [**6**].—Amorphous; uv λ max nm (log ε) 221 (4.06), 274 (3.90), 280 (3.80); ir ν max cm^{-1} 1600, 1460, 1370, 1240, 760; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 310 (71), 295 (16), 267 (42), 214 (37), 203 (100), 174 (12), 163 (41), 145 (8), 133 (30), 107 (88); calcd for $C_{22}H_{30}O$, 310.2297; found (eims) 310.2285.

UVARISESQUITERPENE C [**9**].—Amorphous; uv λ max nm (log ε) 219 (4.18), 273 (3.98), 279 (3.92); ir ν max cm^{-1} 3450, 1600, 1480, 1250, 760; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 328 (4), 310 (4), 285 (46), 256 (49), 241 (26), 203 (7), 197 (18), 179 (12), 149 (100), 145 (4), 123 (25), 107 (19), 73 (6), 55 (32); calcd for $C_{22}H_{32}O_2$, 328.3402; found (eims) 328.2395.

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