EXPERIMENTAL

Aerial portions of *A. michauxii* were dried at 60° for 24 hr, ground to a 40-mesh powder, and stored in a sealed container until use. One-week-old broiler chicks averaging 100 g were used for the toxicity studies. Three chicks were used per treatment. Food and H_2O were removed from the cage at 17.00 so that the crop would be void of food and liquid when the test material was introduced at 08.00 the following day. After the test material was administered, commercial chick feed and water were available free choice. Powdered *A. michauxii* was encapsulated in No. 4 gelatin capsules and administered to the chicks at 1.7% of body weight daily for 2 days. 25 g powdered *A. michauxii* was extracted with 95% EtOH in a Soxhlet. The extract was cooled, filtered, and the filtrate reduced to dryness. The residue was redissolved in 50 ml H_2O, filtered, and the filtrate was extracted with C_6H_6 (×3). The aq. fraction was reduced to 25 ml so that 1 ml of extract was equal to 1 g dried plant. The extract was administered via a rubber catheter into the crops of the chicks in doses of 1, 2, and 3 ml. *A michauxii* was analyzed for nitro concentration by the method of ref. [8] as modified by ref. [9]. 25 g *A. michauxii* was extracted ca 18 hr in cold 80% EtOH. The extract was filtered, evapd to dryness, redissolved in 2 ml 95% EtOH, and spotted, together with an authentic sample of miserotoxin, on a Si gel (250 microns) TLC plate. The plate was chromatographed in C_6H_6–MeOH (5:3). A portion of the crude extract was subjected to preparative pressure chromatography to yield miserotoxin whose NMR and R_f values were identical to an authentic sample. Sprays for developing the plates were: I, 2M NaOH and 95% EtOH (1:1); II, 0.3% soln of *p*-nitroaniline in 1 N HCl and a 5% aq. soln of $NaNO_2$. The TLC plate was sprayed while slightly moist with I followed by II. Nitro compounds present react with II to form red spots.

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AROMATIC CONSTITUENTS FROM *UVARIA CHAMAE*

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Key Word Index—*Uvaria chamae*; Annonaceae; root bark; isolation; *C*-benzylated monoterpene; chamanen; aromatic oils.

Abstract—A novel monobenzylated monoterpene, chamanen, has been isolated from the root bark of *Uvaria chamae*. In addition, the dimethyl ether of thymoquinol, benzyl benzoate, *o*-methoxybenzyl benzoate, *o*-methoxybenzyl benzyl ether and di-*o*-methoxybenzyl ether have been isolated. Structure determinations were accomplished by physical and chemical means.

INTRODUCTION

Recently, the isolation and characterization of three novel *C*-benzylated flavanones chamanetin, isochamanetin, and dichamanetin and three *C*-benzylated dihydrochalcones, uvaretin, isouvaretin, and diuvaretin from *Uvaria chamae* (Annonaceae) were reported [1, 2]. These flavonoids were shown to be responsible for the cytotoxic activity observed in ethanolic extracts of the root bark of *U. chamae*. An investigation of an aromatic oil fraction from the root bark has led to the identification of a novel *C*-benzylated monoterpene, chamanen (**1**), thymoquinol dimethyl ether (**2**), benzyl benzoate, *o*-methoxybenzyl benzoate (**6**), *o*-methoxybenzyl benzyl ether (**7**), and di-*o*-methoxybenzyl ether (**8**).

RESULTS AND DISCUSSION

Silicic acid chromatography of the aromatic oil fraction [2] of the root bark yielded four fractions (A–D). Fraction B gave one major peak on GLC analysis. Purification of this fraction by chromatography over neutral alumina gave thymoquinol dimethyl ether (**2**). The molecular formula ($C_{12}H_{18}O_2$) was established by high resolution MS. The ^1H NMR spectrum of **2** in $CDCl_3$ showed resonances for an isopropyl group (δ1.20, 6H, *d*, *J* 7 Hz and δ3.33, 1H, septet, *J* 7 Hz), an aromatic methyl group (δ2.21, 3H, *s*), and methoxyl groups (δ3.80, δ3.83, 3H ea, *s*) as well as aromatic protons (δ6.70, δ6.77, 1H ea, *s*). The above data are consistent with 2,5-dimethoxy-*p*-cymene (thymoquinol dimethyl ether, **2**).

Treatment of **2** with boron tribromide resulted in conversion to thymoquinol (**3**), mp 141–143° [3].

A portion of fraction D was chromatographed over silica gel and provided four fractions (E–H). Rechromatography of fraction H over neutral alumina gave chamanen (**1**). Chamanen ($C_{18}H_{22}O_2$, high resolution MS) had an ^{1}H NMR spectrum that demonstrated resonances due to an isopropyl group (δ1.17, 6H, *d*, *J* 7 Hz and δ3.27, 1H, septet, *J* 7 Hz), an aromatic methyl group (δ2.23, 3H, *s*), a methoxyl group (δ3.87, 3H, *s*), and a hydroxybenzyl group* (δ3.97, 2H, *s* and δ6.65–7.30, 4H, *m*). The ortho disposition of the hydroxy group in the benzyl substituent was proven by the synthesis of **1** from *o*-hydroxybenzyl alcohol and thymol methyl ether (**4**) using BF_3–Et_2O as catalyst [1, 2]. The signals of the two aromatic protons of the thymol-derived ring of **1** were obscured by the aromatic protons of the hydroxybenzyl moiety. A determination of the substitution pattern of this ring could not be made by ^{1}H NMR analysis†. Therefore, **1** was oxidized with aq. $KMnO_4$. The residue after work up was chromatographed over silica gel. Elution with ether–benzene yielded fractions which were compared by TLC, combined, and then treated with ethereal CH_2N_2. GC–MS analysis of these fractions showed a major component having M^+ 222. Further purification by chromatography over silica gel gave a pure compound having molecular formula $C_{13}H_{18}O_3$ as shown by high resolution MS. The IR and ^{1}H NMR spectra were consistent with the structure shown in **5**. The presence of two 1H singlets in the aromatic region of the ^{1}H NMR spectrum of **5** requires that the methoxy and methyl ester functions be *para* to one another. Thus, the substitution pattern of the thymol-derived rings of **1** and **5** is established.

Fractions C and E both afforded an oil having spectral data consistent with that of benzyl benzoate. Comparison of this oil with an authentic sample of benzyl benzoate confirmed its identity. Fraction G afforded an oil that had spectral data consistent with that of *o*-methoxy-*o*-methoxybenzyl benzoate (**6**). Comparison of this oil with an authentic sample of *o*-methoxybenzylbenzoate confirmed its identity.

Another portion of fraction D was chromatographed over neutral alumina and provided *o*-methoxybenzyl benzyl ether (**7**) and di-*o*-methoxybenzyl ether (**8**), both of which had spectral data consistent with their proposed structures. These two constituents were identified by comparison with authentic samples.

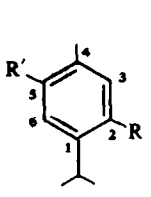
* This aromatic pattern is nearly identical to that of *o*-hydroxybenzyl alcohol [1].

† Even though **1** was synthesized from *o*-hydroxybenzyl alcohol and **4** the substitution pattern is not defined since the *o*-hydroxybenzyl substituent could be located at C-3.

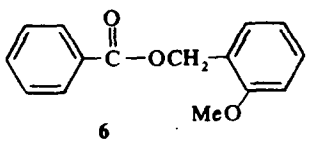
EXPERIMENTAL

^{1}H NMR spectra were recorded at 60 MHz using TMS as internal standard; chemical shift values are reported in δ (ppm) units. GLC was carried out on a 2.43 m column of 3% OV-17. Column chromatography was carried out using Si gel (70–270 mesh) or silicic acid (100 mesh).

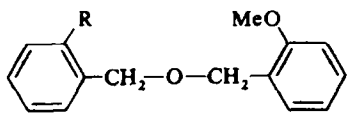
Isolation of aromatic oils. A 10 g portion of the aromatic oil fraction of the root bark of *Uvaria chamae* [2] was dissolved in 10 ml 1:1 C_6H_6–hexane and applied to a column of 250 g silicic acid. Elution with 500 ml 1:1 C_6H_6–hexane yielded an oily fraction, A (1.73 g). Further elution with 150 ml 1:1 C_6H_6–hexane yielded on oily fraction, B (550 mg). Rechromatography of B over 24 g neutral alumina using 1:1 C_6H_6–hexane as eluent yielded 350 mg of thymoquinol dimethyl ether, **2**; IR ($CHCl_3$) ν_{max} 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ6.77 (1H, *s*, Ar-H), 6.70 (1H, *s*, Ar-H), 3.83 (3H, *s*, Ar-OCH$_3$), 3.80 (3H, *s*, Ar-OCH$_3$), 3.33 (1H, septet, *J* 7 Hz, (Me)$_2$–CH-Ar), 2.21 (3H, *s*, Ar-CH$_3$), and 1.20 (6H, *d*, *J* 7 Hz, (CH$_3$)$_2$-CH-Ar); MS: *m/e* 194.1280 (M^+ $C_{12}H_{18}O_2$ requires 194.1307). Further elution with 650 ml C_6H_6 provided an oily fraction, C (3.61 g); IR ($CHCl_3$ ν_{max} 1745, 1610, and 1590 cm^{-1}; ^{1}H NMR (acetone-d_6) δ7.80–8.00 (2H, *m*, Ar-H), 7.10-7.50 (8H, *m*, Ar-H), and 5.26 (2H, *s*, Ar–CH$_2$-OR). A direct comparison of the oil with an authentic sample of benzyl benzoate showed the two samples to be the same (GC, IR, and ^{1}H NMR). Elution with Me_2CO yielded a fourth fraction, D (2.64 g), shown to be a complex mixture by GLC. A 1 g portion of fraction D was chromatographed over 22 g of neutral Al_2O_3. Elution with 10% Et_2O–C_6H_6 yielded 22 mg of an oil, *o*-methoxybenzyl benzyl ether (**7**) IR ($CHCl_3$) ν_{max} 1600 and 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ7.35 (5H, *s*, Ar-H), 6.70–7.50 (4H, *m*, Ar-H), 4.60 (4H, *s*, Ar-CH$_2$-OR), and 3.80 (3H, *s*, Ar-OCH$_3$); MS: *m/e* 228.1145 (M^+ $C_{15}H_{16}O_2$ requires 228.1150). Elution of this Al_2O_3 column with 40% Et_2O–C_6H_6 yielded 35 mg of a gum, di-*o*-methoxybenzyl ether (**8**); IR ($CHCl_3$) ν_{max} 1605 and 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ6.70–7.50 (8H, *m*, Ar-H), 4.60 (4H, *s*, Ar-CH$_2$-OR), and 3.80 (6H, *s*, Ar-OCH$_3$); MS: *m/e* 258.1280 (M^+ $C_{16}H_{18}O_3$ requires 258.1256). Another 1 g portion of fraction D was chromatographed over 21 g silica using C_6H_6 as eluent. Initial C_6H_6 eluates yielded 160 mg of benzyl benzoate followed by a 517 mg mixture of *o*-methoxybenzyl benzyl ether and di-*o*-methoxybenzylether. These fractions were followed by an oily fraction (460 mg) containing *o*-methoxybenzyl benzoate (**6**); IR ($CHCl_3$) ν_{max} 1720, 1610, and 1590 cm^{-1}; ^{1}H NMR (acetone-d_6) δ7.80–8.00 (2H, *m*, Ar-H), 6.70–7.50 (7H, *m*, Ar-H), 5.30 (2H, *s*, Ar-CH$_2$-OR), and 3.88 (3H, *s*, Ar-OCH$_3$).



1: R = OMe; R′ = CH$_2$–C$_6$H$_4$–OH
2: R = R′ = OMe
3: R = R′ = OH
4: R = OMe; R′ = H
5: R = OMe; R′ = CO$_2$Me



6



7: R = H
8: R = OMe

The latter C_6H_6 eluates of this Si gel column contained 95 mg of an oil chamanen (2); IR ($CHCl_3$) ν_{max} 1615 and 1590 cm^{-1}; ^1H NMR (acetone-d_6) δ6.65–7.30 (6H, *m*, Ar-H), 3.97 (2H, *s*, Ar-CH_2-AR), 3.87 (3H, *s*, Ar-OCH_3), 3.27 (1H, septet, *J* 7 Hz, Ar-CH-$(Me)_2$), 2.23 (3H, *s*, Ar-CH_3), and 1.17 (6H, *d*, *J* 7 Hz, $(CH_3)_2$-CH-Ar); MS: *m/e* 270.1627 (M^+ $C_{18}H_{22}O_2$ requires 270.1620.

Demethylation of thymoquinol dimethyl ether (2) *to give thymoquinol* (3). BBr_3 (0.2 ml) was added to a soln of 60 mg 2 in dry CH_2Cl_2 at 0°. The soln was allowed to stand at 0° for 1.5 hr and was then added to 3 ml $NaHCO_3$. This soln was extracted with 3 × 10 ml Et_2O, dried, and evapd. The resulting residue was dissolved in Me_2CO, filtered, evapd, and recrystallized from C_6H_6–EtOH, mp 141–143° [3], ^1H NMR (acetone-d_6) δ6.45 (1H, *s*, Ar-H), 6.37 (1H, *s*, Ar-H), 3.33 (1H, septet-*J* 7 Hz, $(Me)_2$-CH-Ar), 2.17 (3H, *s*, Ar-CH_3), and 1.25 (6H, *d*-*J* 7 Hz, $(CH_3)_2$-CH-Ar); MS: *m/e* 166.0968 (M^+ $C_{10}H_{14}O_2$ requires 166.0994).

Thymol methyl ether (4). Thymol (400 mg) was dissolved in 5 ml N KOH in 20% aq. EtOH. To this soln was added Me_2SO_4 (0.6 ml) in EtOH (0.6 ml). After refluxing 3.5 hr the soln was cooled, evapd, and extracted with Et_2O. Chromatography of this extract over Si gel using C_6H_6 as eluent afforded 350 mg thymol methyl ether (4); ^1H NMR ($CDCl_3$) δ6.70–7.30 (3H, *m*, Ar-H), 3.90 (3H, *s*, Ar-OCH_3), 3.37 (1H, septet, J 7 Hz, Ar-CH-$(Me)_2$), 2.37 (3H, *s*, Ar-CH_3), and 1.23 (6H, *d*, *J* 7 Hz, Ar-CH-$(CH_3)_2$); MS: *m/e* 164.1201 (M^+ $C_{11}H_{16}O$ requires 164.1194).

Synthesis of chamanen (1). A soln of 500 mg *o*-hydroxybenzyl alcohol and 2 ml BF_3–Et_2O in 5 ml dioxane was added over 15 min to a soln of 400 mg thymol methyl ether in 5 ml dioxane at 70°. An additional 2 ml BF_3–Et_2O in 1 ml dioxane was added and the mixture was allowed to stand at 70° for 30 min. The mixture was then diluted, evapd, and chromatographed over 20 g Si gel using C_6H_6 as eluent. Initial eluates contained 200 mg of thymol methyl ether followed by 580 mg of a compound identical to 1 (TLC, co-TLC, IR, and ^1H NMR).

Oxidation of chamanen to give 5. Over a period of 15 min 3 ml of a satd aq. soln of $KMnO_4$ was added to a stirred soln of 100 mg 1 in 2 ml dioxane. The soln was allowed to stir for 30 min more. Excess Na_2SO_3 was added followed by 6N HCl until the soln clarified. The suspension was then extracted with 3 × 20 ml Et_2O. The combined Et_2O layer was dried and chromatographed over 23 g Si gel. TLC analysis (Si gel GF-254, 40% Et_2O–C_6H_6) of the 4, 8, 16, and 32% Et_2O–C_6H_6 eluates showed one major spot. These fractions were combined (15 mg), treated with ethereal CH_2N_2, and then twice chromatographed over Si gel (C_6H_6) to yield 5 mg of 5; IR ($CHCl_3$) ν_{max} 1725, 1620 and 1575 cm^{-1}; ^1H NMR ($CDCl_3$) δ7.80 (1H, *s*, H-6), 6.67 (1H, *s*, H-3), 3.90 (6H, *s*, Ar-OCH_3, Ar-CO_2-CH_3), 3.25 (1H, Ar-CH$(Me)_2$), 2.60 (3H, *s*, Ar-CH_3), and 1.21 (6H, *d*, *J* 7 Hz, Ar-CH$(CH_3)_2$; MS: *m/e* 222.1274 (M^+ $C_{13}H_{18}O_3$ requires 222.1256).

Synthesis of o-methoxybenzyl benzoate (6). Excess benzoyl chloride was added to 50 mg *o*-methoxybenzyl alcohol at 40°. The mixture was shaken and allowed to stand at 40° overnight. The soln was then diluted with 20 ml H_2O and Et_2O. The Et_2O phase was withdrawn and the H_2O phase was extracted with 3 × 20 ml Et_2O. The combined Et_2O phase was dried, evapd, and chromatographed over 20 g Si gel. Using C_6H_6 as eluent 65 mg of a compound identical to 6 (TLC, co-TLC, IR, and ^1H NMR) was obtained.

Synthesis of o-methoxybenzyl benzyl ether (7). o-Methoxybenzyl alcohol (3.0 g) and Na (200 mg) were stirred at room temp. for 6 hr. Benzyl bromide (500 mg) was then added. After stirring for 15 min a white ppt. appeared. The suspension was then acidified to pH 1 with 6N HCl and extracted with 3 × 20 ml Et_2O. The combined Et_2O phase was dried, evapd, and chromatographed over 22 g Si gel. Elution with C_6H_6 yielded 500 mg of a compound identical to 7 (GC, IR and ^1H NMR).

Synthesis of di-o-methoxybenzyl ether (8). *o*-Methoxybenzyl alcohol (2.5 g) and Na (150 mg) were stirred at room temp. for 6 hr. *o*-Methoxybenzyl chloride (300 mg, formed by treatment of *o*-methoxybenzyl alcohol with $SOCl_2$) was added and the soln was allowed to stir overnight. The soln was then acidified and extracted with 3 × 20 ml Et_2O. The combined Et_2O phase was dried, evapd, and chromatographed over 40 g Si gel. Elution with C_6H_6 yielded 280 mg of a compound identical to 8 (TLC, co-TLC, IR and ^1H NMR).

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