Treatment of the acid in benzene with oxalyl chloride followed by reaction with piperidine, filtration and crystallization gave wisanine, (45%) as pale yellow needles, mp 178–180° (EtOAc), λ_{max} (EtOH) (log ε) 249 (4.06), 279 (4.10), 298 (4.10), 366 (4.15) nm, ν_{max} (KBr) 1640, 1610, 1600 cm^{-1}, τ ($CDCl_3$), 2.3–4.0m (6H), 4.15 (2H), 6.3 (3H), 6.5 (4H) 8.45 (6H) (Found: C 68.6; H, 7.1; N, 4.4; $C_{18}H_{21}NO_4$ requires: C, 68.7; H, 6.7; N, 4.5%) showing spectral data closely similar to those for the natural amide, mp 178–179° and mmp 177–179°.

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MISEROTOXIN, A TOXIC COMPOUND IN *ASTRAGALUS MICHAUXII*

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Key Word Index—*Astragalus michauxii*; Leguminosae; miserotoxin; 3-nitro-1-propanol; nitro compounds.

Leaflets of *Astragalus michauxii* (O. Kze.) F. J. Hermann were reported acutely poisonous to broiler chicks when fed at 1.70% of body weight [1]. Toxic signs included depression, ruffled feathers, muscular weakness and incoordination, reduction in body temperature and heart rate, and anorexia. The toxic principle was not identified.

The toxic signs closely resembled those of nitro poisoning produced by feeding nitro-bearing *Astragalus* to chicks [2–4]. The most toxic nitro compound in *Astragalus* is miserotoxin, the β-glucoside of 3-nitro-1-propanol, which was first isolated from *A. miser* var. *oblongifolius* (Rydb.) Cronq. [5]. Subsequent analysis of leaves from herbarium specimens of *A. michauxii* at the New York Botanical Garden, Bronx, NY; University of North Carolina, Chapel Hill; and the University of Georgia, Athens, confirmed the presence of a nitro compound in this species.

The aerial portions of *A. michauxii* were collected in flower and pod in Jenkins, Bulloch, Laurens, and Bleckley Counties, Georgia, on June 11–12, 1976. Voucher specimens (Accession No's. 145,856 and 145,857) are in the Intermountain Herbarium, Utah State University, Logan, Utah. The plant was examined for toxicity to one-week-old chicks when fed as dried plant and aqueous extract, mg NO_2/g of plant, and type of nitro compound present.

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RESULTS

Chicks fed *A. michauxii* at 1.7% of body weight exhibited toxic signs at 24 hr identical with those described previously [1]. Symptoms of toxicity became more pronounced after the second feeding. Twenty-four hr after the second feeding, one bird was paralyzed and comatose; death followed in 5 hr. A second chick became comatose and died 36 hr after the second feeding. The third bird recovered 48 hr after the second feeding. Chicks fed *A. michauxii* extract at 1 ml exhibited no toxic signs. All birds fed 2 ml of extract were depressed, incoordinated, weak, and had ruffled feathers 5 hr after treatment. Two chicks died 8 hr after treatment and the third chick died during the night. Chicks fed 3 ml were affected at 2 hr, comatose at 4 hr, and dead at 6 hr.

A. michauxii analyzed for 10 mg NO_2/g aerial portions and 12 mg NO_2/g leaflets. TLC analysis of *A. michauxii* indicated the presence of only one major nitro compound with the same R_f (0.33) as an authentic sample of miserotoxin. A trace of 3-nitro-1-propanol was indicated at R_f 0.57. A prep scale isolation yielded miserotoxin, whose NMR was identical with that of an authentic sample. The toxic compound in *A. michauxii* is, therefore, miserotoxin.

Taxonomically, *A. michauxii* is the only species within section 43, *Michauxiani*, of Barneby's classification of North American *Astragalus* [6]. *A. michauxii* is thus chemotaxonomically related to *Astragalus* species in sections 40 through 47, many of which are nitro-bearing [7].

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**).