(1)

EXPERIMENTAL

Isolation. The powdered fruits of *P. officinarum* Cas. DC (1.5 kg) were exhaustively extracted with petrol 60–80°. The resultant viscous liquid was adsorbed on a column of neutral Al_2O_3 (Brockmann). Elution with petrol gave a crude yellowish brown liquid which on repeated column chromatography over neutral Al_2O_3 gave a yellowish-white waxy compound, mp 60–62°. Repeated crystallization from petrol furnished a pure white waxy crystalline compound, mp 67–67.5°.

Hydrogenation. The compound (500 mg) in MeOH (375 ml) was hydrogenated over 10% Pd/C catalyst (200 mg) at room temp. and pres. Absorption of H_2 was complete after 1.5 hr during which 3 mol of H_2 was absorbed. The catalyst was filtered, the filtrate evapd under red. pres. to yield a viscous residue which on chromatography over neutral Al_2O_3 yielded a pale yellow crystalline solid, mp 58–60°. Repeated crystallization from petrol-EtOAc furnished a pure (TLC) white crystalline compound $C_{24}H_{49}NO$ (350 mg), mp 72–73°, M^+ 367, (R_f, 0.42, C_6H_6-EtOAc, 1:1).

Hydrolysis of hexahydro derivative. The satd amide (150 mg) was hydrolysed with 10% HCl in EtOH in a sealed tube at 100° for 90 hr. The contents were concd under red. pres., H_2O added and extracted with Et_2O. The aq. portion was concd and the residue after several crystallizations from petrol-EtOAc gave shining colourless plates of *iso*butylamine HCl. mp 170–171° (undepressed with authentic sample). The Et_2O extract was extracted with 5% aq. KOH and the aq. portion acidified and extracted with Et_2O. The Et_2O extract upon removal of the solvent gave a waxy residue. Several crystallizations of the residue from MeOH furnished a white waxy solid, mp 75–76° which was identified as eicosanoic acid by GLC of its Me ester.

Oxidation. $KMnO_4$ (*ca* 1 g) was added in small portions during 30 min to the compound (200 mg) suspended in H_2O (25 ml) and Me_2CO (5 ml) at 50°. The contents were refluxed for 15 min at 100° when the soln became colourless. MnO_2 was removed by filtration and washed with hot H_2O. The filtrate was acidified and steam distilled. *Ca* 200 ml of the distillate was collected and extracted with Et_2O which on evaporation yielded a waxy crystalline compound (35 mg), mp 43.5°, M^+ 200 which was identified as dodecanoic acid by GLC (Me ester) and MS.

Synthesis of hexahydro derivative. Eicosanoic acid (500 mg) in dry C_6H_6 on treatment with freshly dist. $SOCl_2$ (2.5 ml) gave the corresponding acid chloride. *Iso*butylamine (1.5 ml) dissolved in dry C_6H_6 was added slowly with stirring at 0–5° to the acid chloride in dry C_6H_6. Removal of solvent under red. pres. gave a thick liquid which on chromatography over neutral Al_2O_3 and crystallization from petrol-EtOAc afforded the *iso*butylamide of eicosanoic acid as a white crystalline compound mp 71–73°, identical with the hydrogenated product (TLC, mmp IR and PMR).

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SYNTHESIS OF WISANINE, A NEW PIPERINE AMIDE FROM *PIPER GUINEENSE*

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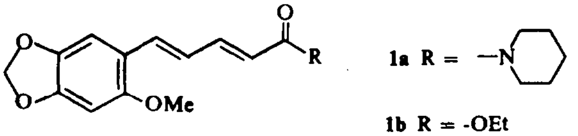
Key Word Index—*Piper guineense*; wisanine; piperine amide.

Wisanine is a new piperine-type amide isolated recently together with the (α,β)-dihydro analogue from seed and roots of the W. African black pepper *Piper guineense*. Structure **1a** has been proposed for the amide [1, 2]. In connection with our contemporaneous synthetic studies amongst piperine alkaloids from other species of *Piper*, we now record a synthesis of wisanine.



1b R = -OEt
1c R = -OH

Condensation between 2-methoxypiperonal [3] and 3-ethoxycarbonylbut-2-enyltriphenylphosphorane led first to the *trans,trans*-diene ester (**1b**) (70%), yellow needles, mp 64–66° (benzene) λ_{max} (EtOH) 238, 271, 309, 370 nm, ν_{max} (KBr) 1700, 1620, 1610 cm^{-1}, τ 2.0–4.2 m (6H), 4.1 (2H), 5.8 *q* (*J* 7, 2H), 6.25 (3H), 8.7*t* (*J* 7, 3H) (Found: C, 65.3; H, 6.1. $C_{15}H_{16}O_5$ requires: C, 65.2; H, 5.8%) which was then saponified to the corresponding acid (**1c**) (67%), yellow microcrystals, mp 220–223° (benzene) (naturally derived acid shows mp 221–224°), λ_{max} (EtOH) (log ε) 244 (4.06), 261 (4.02), 269 (4.02), 300 (4.0), 368 (4.13) nm, λ_{max} (KBr) 1690, 1620, 1608 cm^{-1} τ (CD_6CO) 2.0–4.0m (6H), 4.1 (2H), 6.0 (3H) (Found: C 62.7; H, 4.8. $C_{13}H_{12}O_5$ requires: C, 62.9; H, 4.8%).

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.

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].

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