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N-ISOBUTYL-*TRANS*-2-*TRANS*-4-EICOSADIENAMIDE AND OTHER ALKALOIDS OF FRUITS OF *PIPER GUINEENSE**

IVAN ADDAE-MENSAH†, FRANK G. TORTO†, ISAAC V. OPPONG†, IAN BAXTER‡§ and JEREMY K. M. SANDERS‡

†Department of Chemistry, University of Ghana, Legon, Ghana; ‡University Chemical Laboratories, Lensfield Road, Cambridge CB2 1EW, England

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Key Word Index—*Piper guineense*; Piperaceae; alkaloids; *N*-isobutyl-*trans*-2-*trans*-4-eicosadienamide; dihydropiperine *N*-piperidyl-5-(3,4-methylenedioxyphenyl)-*trans*-2-pentamide.

Abstract—A novel alkaloid, *N*-isobutyl-*trans*-2-*trans*-4-eicosadienamide, has been isolated from the fruits of *Piper guineense* and fully characterized. The structure of the compound has been confirmed by an unambiguous synthesis of the tetrahydro derivative. The known alkaloid, $\Delta\alpha\beta$-dihydropiperine has also been isolated and the position of the double bond in this compound confirmed by the use of an NMR shift reagent.

INTRODUCTION

The genus *Piper* has attracted much interest in recent times because of the number of physiologically active compounds it contains [1, 2]. *Piper guineense*, West African Black Pepper or Ashanti Pepper, is a wild or semicultivated climbing plant widely used as a medicinal plant [3] and as a spice. The fruits have been reported to contain lignans [4, 5], and some amide alkaloids [6–8]. Okogun *et al.* [7] isolated a mixture of *N*-isobutyl-*trans*-2-*trans*-4-dienamides, which after hydrogenation, was shown by GLC and MS to be *ca* 75% *N*-isobutyl steramide. The isobutylamides of palmitic and eicosanoic acids were said to be present in the mixture only in trace amounts detectable by MS. At the time of this report, we had already isolated *N*-isobutyl-*trans*-2-*trans*-4-eicosadienamide (**1**), not as a minor component, but as the third most abundant constituent of the fruits after trichostachine [9] and piperine [7]. We report here the isolation and characterization of this compound in the fruits, as well as the presence of other known piper alkaloids.

RESULTS AND DISCUSSION

The petrol extract of the powdered dry fruits was separated into three fractions (X, Y, and Z).

From fraction Z was isolated a white crystalline material which decolourized Br_2 in CCl_4, and gave a negative Labat's test for a methylenedioxy group. The UV λ_{max} (EtOH) 262 nm, indicated a —CH=CH—CH=CH—CON system [10]. Exact mass determination and elemental analysis gave the molecular formula $C_{24}H_{45}NO$. The IR spectrum showed absorptions at 3375 (CONH) 1690 and 1660 (amide CO), α,β-unsaturated amide absorptions at 1642 and 1580. and a *trans*-CH=CH— bond absorption at 1010 cm^{-1}. The PMR spectrum and MS fragmentation pattern were consistent with the proposed structure (**1**). The MS also showed a prominent peak at *m/e* 307 which could have arisen through a McLafferty-type rearrangement during fission of the NH—CH_2-bond [11].

Hydrogenation of the compound gave the *N*-isobutylamide of eicosanoic acid (**2**) M^+ at *m/e* 367, $C_{24}H_{49}NO$. The IR spectrum showed a clear absence of a *trans*-substituted —CH=CH— peak at 1010 cm^{-1}. Other spectral data (MS, PMR) were consistent with the structure of the compound. Hydrolysis of (**2**) with 10% ethanolic KOH [12] gave eicosanoic acid, identical with authentic material.

Column chromatography of X gave 6 main fractions. Fraction (i), an oil (6 g) was shown by GLC to contain about 6 different compounds. Fraction (ii) on standing for several weeks in EtOAc at 0°, deposited a pinkish-white solid which recrystallized (EtOAc) to give white crystals, $C_{17}H_{21}NO_3$. The UV spectrum λ_{max} (EtOH) 235, 290, 347 (*sh*) indicated either a methylenedioxy styryl or a —CH=CH—CO chromophore. Hydrogenation (EtOH, prereduced Adam's catalyst) gave a product identical (UV and IR) with tetrahydropiperine. The IR of the natural compound showed the presence of an amide carbonyl group as well as a *trans*-CH=CH— bond. The PMR spectrum showed peaks at δ 1·60 (6H, *s*, $—CH_2)_3$— of piperidine ring); 2·40–2·78 (4H, *m*, —$\underline{C}H_2$—$\underline{C}H_2$—CH=CH—); 3·50 (4H, *br*, —$\underline{C}H_2$—N—$\underline{C}H_2$— of piperidine ring); 5·90 (2H, *s*, —OCH_2O—) (6·18 (1H, *d*, J = 8 Hz); 6·54–6·84 (4H, *m*, including a 1H doublet, J 16 Hz) *trans*-CH=CH— and 3 aromatic protons. The above data suggested that the structure of this alkaloid could be (**3**) or (**4**).

Loder *et al.* isolated a gum from *P. Novae-Hollandiae*, and on the basis of UV, MS and synthetic evidence, assigned structure (**4**) to it. The UV spectrum of the synthetic compound showed λ_{max} at 235, 285 [2]. Dwuma-Badu *et al.* also recently isolated the same compound from the roots of *P. guineense*, and characterized it by direct comparison with Loder's synthetic compound [8]. However, the method of synthesis was not unambiguous. It involved reduction of piperic acid with

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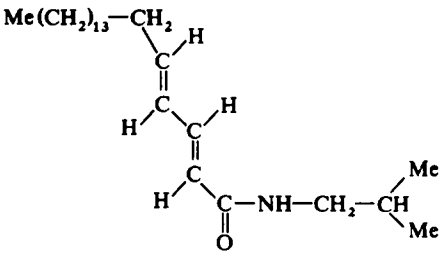
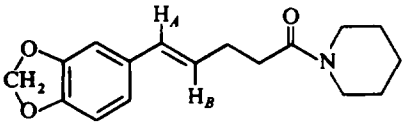
§ Present address: Careers and Appointments Advisory Centre, University of Edinburgh, Edinburgh EH8 9JT, Scotland.

Na–Hg in EtOH, followed by conversion of the resulting dihydro derivative to the acid chloride, and treatment of this with piperidine. But there is no reason why the



(1)

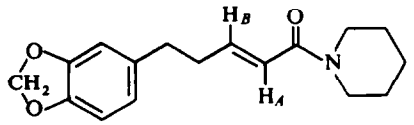
reaction should result in preferential reduction of the double bond conjugated to the benzene ring, as opposed to the one conjugated to the carbonyl group of piperic acid. Systems of the type RO—Ar—CH=CH—COOR' (where RO is either a methoxy or methylenedioxy group) and Ar—CH=CH—CH—CH=CH—$CONR_2$ can have their carbonyl group reduced by much stronger reducing agents (e.g. $LiAlH_4$) without affecting the styryl double bond [13]. Therefore even though the UV spec-



(3)

trum of Loder's compound and ours were more similar to each other than to that of either methylenedioxy cinnamic acid or 3-(3,4-methylenedioxy phenyl)-2-propene [14], it was necessary to determine unambiguously the position of the double bond by some other means. This was done using the NMR shift reagent $Eu(DPM)_3$.

In the presence of the shift reagent (4.4 mg reagent added to 0.09 M $CDCl_3$ soln of alkaloid), the axial and equatorial protons adjacent to N in the piperidine ring



(4)

became non-equivalent and appeared as two broad peaks at δ3.95 and 4.52. This indicated that complexation occurred at the amide group. The spectrum also showed a very fast alkenic proton shift for proton H_A, from 6.18 (*d*) to 6.95 (*d*). The chemical shifts for the methylene protons H_C and the methylenedioxy group were virtually unaffected. This indicated that proton H_A was adjacent to the C=O group where complexation occurred, and not to the aromatic ring.

Moreover, the MS of our compound showed a base peak at *m/e* 135, indicative of a methylenedioxy tropylium cation, which is consistent with a CH_2 group and not a —CH=CH— group attached to the benzene ring [15, 16]. The above evidence, as well as the MS fragmentation pattern based on that postulated for similar systems [2, 15], supported structure (4), *N*-piperidyl-5-(3, 4-methylenedioxy phenyl)-*trans*-2-pentenamide ($\Delta\alpha\beta$-dihydropiperine).

The other fractions from the column gave mainly piperine and trichostachine.

EXPERIMENTAL

Plant material was collected from Gbadzeme, a village in the Volta Region of Ghana, in June–July. Voucher specimens of the plant have been deposited at the Herbarium of the Botany Department, University of Ghana, Legon.

N-Isobutyl-trans-2-trans-*4-eicosadienamide* (1). Dried powdered fruits (3.5 kg) were Soxhlet extracted for 24 hr with petrol (bp 60–80°). Extract was left in refrigerator to deposit a reddish oily mixture (X, 120 g). The supernatant liquid on refrigeration deposited a crude yellow solid (Y, 22 g). The mother liquor from Y was concentrated and refrigerated to deposit a yellowish-white solid (Z). Z was triturated several times with cold petrol, purified by preparative-TLC (Si gel, cyclohexane–$CHCl_3$, 3:1), recrystallized (EtOAc) to give white crystals (0.79 g, 0.02%), mp, 89–90°. UV λ_{max} (EtOH) 262 nm (log ε 4.81). Exact mass determination M^+ at *m/e* 363.3483, (calc 363.3500), $C_{24}H_{45}NO$. IR ν_{max} (KBr) 3375, 1690, 1660, 1642, 1580, 1010 cm^{-1}. PMR (100 MHz, $CDCl_3$): δ 0.87 (6H, *d*, *J* = 7 Hz, —CH—($\underline{CH_3}$)$_2$; 0.93 (3H, *t*, partly obscured by —$(CH_2)_n$— singlet, ($\underline{CH_3}$—$(CH_2)_n$—); 1.26 (13H, *s*, —Me—($\underline{CH_2}$)$_{13}$—); 1.54 (1H, *m*, —CH_2—$\underline{CH}$—$(Me)_2$); 2.06–2.28 (2H, *m*, $(CH_2)_{13}$—$\underline{CH_2}$); 3.12 (2H, *t*, *J* = 7.5 Hz, —NH—$\underline{CH_2}$—); 5·69–6.20 (1H, *m*, —CH=$\underline{CH}$—CON—); 7.02–7.32 (3H, *m*, *trans*-CH_2—$\underline{CH}$=$\underline{CH}$—$\underline{CH}$=CH—). MS fragments *m/e* (%) 363 (M^+), 362 (24.1), 348 (30.6), 320 (75.3), 307 (43.5), 291 (98.9), 263 (97.7), 81 (100). Found: C, 79.5; H, 12.4; N, 3.9%. $C_{24}H_{45}NO$ requires C, 79.3; H, 12.4; N, 3.9%.

N-Isobutyl eicosanamide (2) *from naturally occurring dinenamide. N*-isobutyl-*trans*-2-*trans*-4-eicosadienamide (0.23 g) was hydrogenated (EtOH, prereduced Adam's catalyst). Recrystallization from EtOH gave the title compound as white crystals (0.21 g), mp 77–78°. M^+ at *m/e* 367. PMR (100 MHz, $CDCl_3$); δ 0.88, 0.96 (9H, two *s*, $\underline{CH_3}$ —$(CH_2)_n$ and CH—($\underline{CH_3}$)$_2$ resp), 1.29 (34H, *s*, Me($\underline{CH_2}$)$_{17}$)); 1.5 (1H, *m*, —$\underline{CH}$ $(Me)_2$); 2.2 (2H, *t*, *J* = 6 Hz; —$\underline{CH_2}$—CON—); 3.1 (2H, *t*, *J* = 6Hz, —C—$\underline{CH_2}$ —N—). IR ν_{max} (KBr), 3375 (—NH—) 3000, 1670 (secondary amide CONH). Found: C, 78.9; H, 13.2; N, 4.1%. Calc for $C_{24}H_{49}NO$: C, 78.5; H, 13.3; N, 3.8%.

Synthesis of N-*Isobutyl eicosanamide.* Eicosanoic acid (0.5 g) was converted to the acid chloride by the conventional method. The resulting product was refluxed with isobutylamine (0.5 g) in dry C_6H_6 for 3 hr. Reaction mixture was left in refrigerator to deposit the title compound (0.3 g), identical (mp, mmp, IR, PMR, MS) with the hydrogenated natural product. Found: C, 78.5; H, 13.0; N, 4.3%. Calc for $C_{24}H_{49}NO$: C, 78.5; H, 13.3; N, 3.8%.

N-*Piperidyl-5-(3,4-methylenedioxy phenyl)*-trans-*2-pentenamide* ($\Delta\alpha\beta$-*dihydropiperine*). Fraction X (50 g) from the petrol extract was chromatographed on Al_2O_3 (neutral), using cyclohexane, cyclohexane–$CHCl_3$, and $CHCl_3$ as eluting solvents. The 2nd of 6 fractions obtained from the column, was left to stand for several weeks in EtOAc in the refrigerator. This deposited a pinkish-white solid which was recrystallized several times from EtOAc to give white crystals (0.3 g) mp 79–80°. MS; M^+ at *m/e* 287, λ_{max} (EtOH) 235, 290, 347 (*sh*), (log ε 4.10, 3.78, 3.04). IR ν_{max} (KBr) 1680 cm^{-1} (amide C=O), 1010 cm^{-1} (trans-CH=CH—). PMR spectrum as indicated in text. Found: C, 70.9; H, 7.4; N, 4.8%. Calc for $C_{17}H_{21}NO_3$: C, 71.1; H, 7.3; N. 4.9%.

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