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NOVEL AMIDE ALKALOIDS FROM THE ROOTS OF *PIPER GUINEENSE**

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Key Word Index—*Piper guineense*; Piperaceae; alkaloid; wisanine; *N*-piperidyl-5-(2-methoxy-4,5-methylenedioxy-phenyl-*trans*-2-*trans*-4-pentadienamide; wisanidine; *N*-pyrrolidyl-5-(2-methoxy-4,5-methylenedioxyphenyl)-*trans*-2-*trans*-4-pentadienamide; *N*-isobutyl-*trans*-2-*trans*-4-eicosadienamide; piperine; $\Delta^{\alpha,\beta}$-dihydropiperine.

Abstract—Two novel amide alkaloids, wisanine and wisanidine, have been isolated from the petroleum extract of the roots of *Piper guineense*, and found to be *N*-piperidyl-5 (2-methoxy-4,5-methylenedioxyphenyl)-*trans*-2-*trans*-4-pentadienamide and *N*-pyrrolidyl-5-(2-methoxy-4,5-methylenedioxyphenyl)-*trans*-2-*trans*-4-pentadienamide respectively. The structure of wisanidine has been confirmed by synthesis. *N*-Isobutyl-*trans*-2-*trans*-4-eicosadienamide, recently reported to be present in the fruits of the plant, as well as Piperine and $\Delta^{\alpha,\beta}$-dihydropiperine have also been found to be major constituents of the roots.

INTRODUCTION

Piper species have been found to contain a number of physiologically active compounds [1, 2], mainly amide alkaloids such as piperine. *Piper guineense*, the West African Black Pepper or Ashanti Pepper, has only recently been investigated. The medicinal uses of the plant include its use for treating various venereal diseases and intestinal disorders [3].

The fruits of *P. guineense* have been reported to contain lignans [4, 5, 16] and some amide alkaloids [6–9]. As part of our investigation of this plant, we recently reported the presence of a novel alkaloid to which we gave the trivial name wisanine [10] (the plant is known by the Akans of Ghana as Osoro Wisa or Sasema). This paper describes details of the isolation and characterisation of this alkaloid, which is the major constituent of the roots, but has so far not been found in the fruits. We also report the isolation and characterisation of another new alkaloid, wisanidine, the pyrrolidine analogue of wisanine, and whose structure has been confirmed by synthesis.

RESULTS AND DISCUSSION

The petroleum extract of the ground root bark deposited an oily semi-solid from which wisanine was isolated. It crystallised from ethyl acetate as large yellow prisms, mp 179–181°. It gave a positive Labat's test for a methylenedioxy group. The UV spectrum indicated a piperine-type chromophore [11, 12]. The IR spectrum showed peaks corresponding to a tertiary amide carbonyl, *trans* conjugated alkenic double bonds, and a methylenedioxy group. Exact mass determination and elemental analysis gave a molecular formula $C_{18}H_{21}O_4N$. The NMR showed absorptions similar to that of piperine [13] and also a sharp singlet at δ 3.71 corresponding to an aromatic methoxy group. On the basis of the above evidence, wisanine was tentatively assigned the structure (**1**).

Significant peaks in the MS could be accounted for in terms of fragmentation at the N—CO bond, followed by cyclisations, as postulated by Chatterjee and Dutta [12], and by Loder *et al.* [2]. For the cyclizations to occur, the methoxy group could be in the 2, 3 or 6 positions. Biosynthetic considerations would favour the 3-position, with the methylenedioxy groups in the 4 and 5 positions, assuming the compound were derived from gallic acid, but if it were formed by methoxylation of piperic acid, then substitution in the 2 or 6 position is also possible. To determine the postion of the methoxy group, the compound was hydrolysed to give wisaninic acid (**2a**), mp 221–224°. This was then hydrogenated to give the tetrahydro derivative (**3**), mp 105–106°, analysing for $C_{13}H_{16}O_5$. The NMR spectrum of (**3**) showed, among other significant peaks, two sharp singlets in the aromatic region, indicating two para protons. These results confirmed the structure of the acid (**2a**), which was supported by the MS fragmentation pattern. On the basis of the above evidence wisanine was formulated as *N*-piperidyl-5-(2-methoxy-4,5-methylenedioxyphenyl)-*trans*-2-*trans*-4-pentadienamide (**2b**). This was consistent with the mass spectral fragmentation pattern, [10] based on that postulated for similar systems by Chatterjee and Dutta [12], and by Loder *et al.* [2].

Wisandine was obtained as a minor component. It crystallised as yellow needles from ethyl acetate/diethyl ether, mp 171–173°. It gave a positive Labat's test for a methylenedioxy group. Its UV spectrum showed peaks at λ_{max} (EtOH) 252, 304, 308, and 378 nm, similar to that reported for wisanine [10]. The IR spectrum showed a tertiary amide carbonyl peak at 1660, and 1610, as well as peaks corresponding to *trans*-conjugated alkenic

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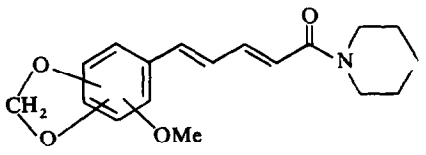
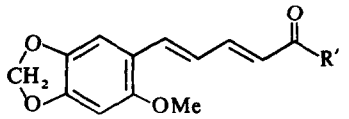


(1)

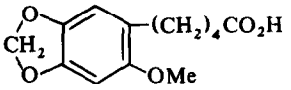


(2)

2a; R′ = OH

2b; R′ = N

2c; R′ = N



(3)

double bonds and a methylenedioxy-phenyl group. The MS gave M^+ at *m/e* 301, and the compound analysed for $C_{17}H_{19}O_4N$. The NMR spectrum showed the presence of a methoxy group as well as signals similar to those reported for trichostachine [11].

Hydrolysis of the compound gave an acid which was identical (MP, mixed MP, UV, IR) with (**2a**), indicating that the methoxy group of wisanidine was in the same position as that of wisanine. This was consistent with the MS fragmentation pattern which showed a peak at 201, corresponding to the loss of an *ortho*substituted OMe group. It has been shown that cinnamic acid derivatives readily lose an *ortho* substituent, presumably by cyclisation to yield a pyrillium ion [14]. On the basis of the above evidence, wisanidine was formulated as *N*-pyrrolidyl-5-(2-methoxy-4,5-methylenedioxyphenyl)-*trans*-2-*trans*-4-pentadienamide (**2c**). The structure was confirmed by synthesising the compound by converting wisaninic acid (**2a**) to the acid chloride, and treating this with pyrrolidine. The synthetic material was identical in all respects (mp, mixed mp, IR, UV, NMR and MS) with the natural compound. Since the completion of this work, it has come to our notice that Okogun *et al.* have also isolated wisanine and its dihydro derivative from the Camerounian variety of the same plant, but not from the Nigerian variety [15]. No wisanidine has been detected in either of the two varieties, neither has the dihydro derivative of wisanine been obtained from the Ghanaian variety. As far as we are aware, these results are the first reports of piperine-type alkaloids containing both a methoxy and a methylenedioxy group in the same benzene ring. Since *Piper* species have been reported to show variation of constituents with the geographical location of the plant [4, 5, 16], the above results may be biogenetically and chemotaxonomically significant.

Column chromatography of the combined mother liquors obtained after isolation of wisanine and wisanidine, gave *N*-isobutyl-*trans*-2-*trans*-4-eicosadienamide (**4**) mp 89–90°, characterised by direct comparison (mp, mmp, UV, IR, hydrolysis, hydrogenation) with material isolated from the fruits and fully characterised [9]. The column also yielded a crystalline compound, mp 149–151°, found by gas chromatography and MS. to be a mixture of three sterols, $C_{29}H_{50}O$, $C_{29}H_{48}O$, and $C_{28}H_{48}O$.

We made three separate collections of plant material from the same location, at different periods; June–July 1974, January–February 1975 and September–October 1975. The collections in June–July, and September–October, both yielded wisanine, wisanidine and *N*-isobutyl-*trans*-2-*trans*-4-eicosadienamide, as well as the sterols. However, the collection made in January–February, when the plants in that locality had either just passed their fruiting stage or had immature fruits, did not yield any wisanine or wisanidine even in trace amounts. They however yielded large amounts of piperine, $\Delta^{\alpha,\beta}$-dihydropiperine, and *N*-isobutyl-*trans*-2-*trans*-4-eicosadienamide. We have observed similar variations in the fruits [17]. It therefore appears that there is, in addition to variation of constituents with change in location, a seasonal variation of constituents.

EXPERIMENTAL

The roots were collected from Fume, a village in the Volta Region of Ghana, in June–July, September–October, and January–February. Voucher specimens of the plant have been deposited at the Herbarium of the Botany Department, University of Ghana, Legon.

Wisanine (**2b**). Dried powdered roots (4.3 kg) were extracted with petrol (bp 60–80°) for 48 hr. The extract was concd and left in the refrigerator for several weeks to deposit an oily semi-solid which was then washed with cold MeOH until the washings were no longer coloured yellow. The MeOH fraction was evaporated to dryness under vacuum and triturated with Et_2O. The ether fraction deposited a yellow solid almost immediately, but was left in the refrigerator to deposit more solid (11.5 g). TLC on Si gel ($CHCl_3$–cyclohexane 3:1) showed two major spots and several minor spots. The faster running major spot was separated by preparative TLC ($CHCl_3$), recrystallised from EtOAc–Et_2O to give yellow prismatic crystals of wisanine, mp 179–181°. λ_{max} (EtOH) 250, 304, 309, 364 (log ε 4.00, 4.16, 4.12, 4.32 nm). MS M^+ at *m/e* 315 (68%). Other significant peaks (%) 231 (100, M^+-piperidine), 203 (11.4; 231-CO), 202 (22.7; 203-H), 172 (45.5; 203-CH_2O), 145 (31.8; 173-CO), 115 (31.8; 145-CH_2O). Exact mass determination M^+ 315.1476 (calculated for $C_{18}H_{21}O_4N$, 315.1470). Found: C, 68.5; H, 6.8; N, 4.3%. $C_{18}H_{21}O_4N$ requires: C, 68.6; H, 6.7; N, 4.4%. IR (KBr) ν_{max} *1640, 1630, 1600* (Unsaturated amide), *1020* (*trans* R—CH=CH—R′) 1280, 940 ($PhCH_2O$). NMR (80 M Hz, $CDCl_3$, deuterium lock); δ 1.55 (6H, *s*, $—CH_2CH_2CH_2—$ of piperidine ring), 3.46 (4H *br*, $—CH_2NCH_2—$ of piperidine ring) 3.71 (3H, *s*, ArOMe), 5.84 (2H, *s*, $—OCH_2O—$), 6.0–7.7 (6H, *m*, two aromatic and four olefinic protons).

Wisaninic acid (**2a**). Wisanine (0.7 g) was refluxed for 24 hr with 10% alcoholic KOH (90 cm^3). The soln was acidified with dil. HCl and the resulting yellow ppt. recrystallized (MeOH) to give the title compound (0.34 g), mp. 221–224°. MS. M^+ at *m/e* 248. IR (KBr) 3400–3000 (*br*) 1740 (*s*) (COOH). NMR spectrum consistent with structure.

Tetrahydrowisaninic acid) (**3**). Wisaninic acid (0.21 g) in 95% EtOH was hydrogenated (prereduced palladium catalyst) until no more hydrogen was taken up. The oily residue obtained after evaporation was recrystallised (dil. EtOH) to give the title compound (0.15 g) mp 105–106°. Found: C, 61.7; H, 6.6%. $C_{13}H_{16}O_5$ requires: C, 61·7; H, 6.4% NMR (60 M Hz, $CDCl_3$, TMS): δ 1.5 (4H, *m*, $—C—CH_2—CH_2—C—$), 2.3 (4H, *m*, $—CH_2Ar$ and $—(CH_2COO—$), 3.7 (3H, *s*, $ArOCH_3$), 5.84 (2H, *s*, $—OCH_2O—$), 6.46, 6.58 (2H, *s*, two *p*-substituted aromatic protons). MS M^+ at *m/e* 252 (68%). Other significant peaks (%) 165 (100, M^+-$(CH_2)_3$ COOH, as tropylium species); 152 (59.7, M^+-$(CH_2)_4$ COOH); 135 (58.3, 165-OMe, as methylenedioxy-tropylium cation).

Wisanidine (**2c**). TLC separation of the second slower running major spot occurring with wisanine, gave wisanidine as yellow crystals from EtOAc–Et_2O, mp 171–173°. λ_{max} (EtOH) 252, 304, 308, 378 nm (log ε 4.06, 4.21, 4.20, 4.38), IR (KBr) ν_{max} 1660, 1600 (unsatd *tert.* amide) 1020 (*trans*-R—CH=CH—R^1) 1290, 960 (Ph OCH_2O). MS: M^+ at *m/e* 301 (23.5%). Other significant peaks (%) 231 (23.5; M^+-pyrrolidine); 201 (13.1; 231-CH_2O) 173 (9.3; 201-CO) 172 (5.0; 173-H or 203-CH_2O). 145 (5.5; 173-CH_2O) 117 (1.1; 145-CO) 115 (10; 145-CH_2O) 83 (100%), Found: C, 67.9; H, 6.4; N, 4.9%; $C_{17}H_{19}O_4N$ requires: C, 67.7; H, 6.3; N, 4.7% NMR ($CDCl_3$) δ: 1.81–2.05 (4H, *m*, —CH_2—CH_2— of pyrrolidine ring); 3.55 (4H, *t*, J = 7 Hz, —CH_2—N—CH_2— of pyrrolidine ring); 3.76 (3H, *s*, ArOMe); 5.90 (2H, *s*, Ph $\underline{OCH_2}O$) 6.22 1H, *d*, J = 14 Hz, *trans*-CH=$\underline{CH}$—CO) (6.52, 1H, *s*, C_3 aromatic proton), 6.65–7.65, 4H, m, aromatic proton and three alkenic —$\underline{CH{=}CH{-}CH}$ = CH—CO protons.

Hydrolysis of wisanidine. Wisanidine (0.05 g) was refluxed for 24 hr in ethanolic KOH and treated as was done for wisanine to give the acid (0.02 g) mp, 217°, mixed mp, IR, and UV identical with authentic material.

Synthesis of wisanidine. Wisaninic acid (0.2 g) in dry C_6H_6 was treated at 0° with $SOCl_2$ (3 ml). The mixture was then refluxed for 1 hr and left at room temp. overnight. Excess $SOCl_2$ was removed by distillation with dry C_6H_6, and the residue redissolved in dry C_6H_6. This was then treated with an excess of pyrrolidine in dry pyridine and left at room temp. overnight. The reaction mixture was then diluted with more C_6H_6, washed with dil. HCl, aq. Na_2CO_3, and H_2O. The acid and base washings were re-extracted with $CHCl_3$, and the organic layer washed with H_2O. The 2 organic fractions were then dried, combined, and purified by TLC (Si gel, $CHCl_3$ as solvent) and recrystallised from EtOAc–Et_2O to give yellow needles mp 169–171°, mixed mp with natural compound undepressed. UV, IR, NMR and MS identical in all respects with natural compound. Found: C, 67.5; H, 6.4; N, 4.7%. Calcd for $C_{17}H_{19}O_4N$: C, 67.7; H, 6.3; N, 4.7%.

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