

## Extracts from the Fruits of *Piper guineense* Schum. and Thonn

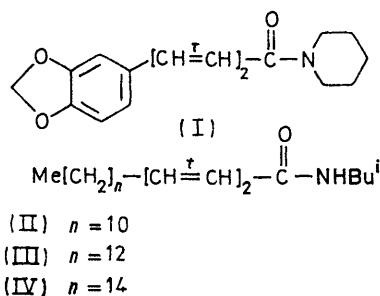
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Extraction of the fruits of *P. guineense* with petroleum has yielded the isobutylamides of 13-(3,4-methylenedioxyphenyl)-2,4,12-tridecatricienoic, 13-(3,4-methylenedioxyphenyl)undeca-2,4,12-trienoic, eicosa-2,4-dienoic, octadeca-2,4-dienoic, and hexadeca-2,4-dienoic acids, as well as the known amides piperine, trichostachine, and piperlonguminine, and a mixture of unidentified sterols.

*PIPER GUINEENSE* (Piperaceae) is a climber which is semi-cultivated in the forest regions of Nigeria. It is commonly used as an ingredient in local medicinal preparations and as a spice in foods. The genus *Piper* has been widely investigated chemically<sup>1-4</sup> and shown to contain piperine (I) type amides. The fruits of *Piper guineense* have been shown to exhibit<sup>5</sup> variation in their chemical composition dependent on geographical location, and to contain piperine and its isomers as well as a number of lignans. We have examined extracts of the fruits of *P. guineense* collected in the Western parts of Nigeria and found sesamin and three known piperine-type amides, as well as five new amides.

Extraction of the fruits with light petroleum and with chloroform gave qualitatively similar mixtures of compounds from which the following fractions were obtained on chromatography on activated alumina.

Fraction (i) was a mixture of the *N*-isobutyl-*trans*-



2,trans-4-dienamides (II)—(IV). Its i.r. and u.v. spectra were characteristic of the systems<sup>6</sup>  $-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$ ,  $\text{CH}\cdot\text{CO}\cdot\text{NHR}$ , and  $-\text{[CH}_2\text{]}_n-$ . Its <sup>1</sup>H n.m.r. spectrum showed absorptions (a) in the vinyl region similar to those of piperine,<sup>7</sup> (b) characteristic of the isobutyl group, and (c) due to a polymethylene system. The mass spectrum showed intense molecular ion peaks at  $m/e$  335 and 307 and a weak peak at  $m/e$  363. Significant fragment ion peaks were present at  $m/e$  263 and 235, corresponding to the fragments ( $\kappa$ )<sup>4</sup> from (II) and (III), respectively.

Complete catalytic hydrogenation of the mixture gave crystals with an i.r. spectrum almost identical with those of the *N*-isobutylamides of palmitic and stearic acids and lacking any u.v. absorptions. G.l.c.—mass spectrometry of the hydrogenated mixture indicated the presence of

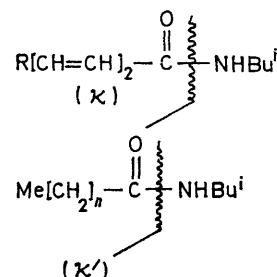
<sup>1</sup> R. Fittig and W. H. Mielck, *Annalen*, 1874, **172**, 134; R. Fittig and L. Winstein, *ibid.*, 1885, **227**, 31.

<sup>2</sup> F. S. Spring and J. Stark, *J. Chem. Soc.*, 1950, 1177.

<sup>3</sup> K. L. Dhar and C. K. Atal, *Indian J. Chem.*, 1967, **5**, 588.

<sup>4</sup> J. W. Loder, A. Moorhouse, and G. B. Russell, *Austral. J. Chem.*, 1969, **22**, 1531.

three amides,  $M^+$  367, 339, and 311, corresponding respectively to the isobutylamides of eicosanoic, stearic, and palmitic acids. Each spectrum possessed a peak characteristic of the fragment ( $\kappa'$ ).<sup>4</sup> The mass spectrum of the mixture again showed a weak peak at  $m/e$  367 but very intense peaks at  $m/e$  339 and 311. The spectra of



the components that had  $M^+$  339 and 311 were identical, respectively, with those of the *N*-isobutylamides of stearic and palmitic acids, and had the characteristic peak arising from cleavage<sup>4</sup> of the C-NH bond. G.l.c. showed that the mixture was about 75% *N*-isobutylstearamide.

The mixture of saturated amides was hydrolysed with methanolic potassium hydroxide and the resulting acids were esterified with ethereal diazomethane. The mixture of esters, analysed by g.l.c.—mass spectra was separated into compounds showing  $M^+$  326, 298, and 270, corresponding respectively to methyl eicosanoate, methyl stearate, and methyl palmitate.

Fraction (ii) was sesamin.<sup>5,8,9</sup>

Fraction (iii) was a compound,  $\text{C}_{24}\text{H}_{33}\text{NO}_3$ ,  $M^+$  383.2465, which we name guineensine. Its i.r. spectrum showed the presence of an unsaturated amide, a *trans*-disubstituted double bond, a styryl double bond, and a methylenedioxy-group.<sup>10</sup> Its <sup>1</sup>H n.m.r. spectrum showed absorptions similar to those of the *N*-isobutyl-*trans*-2,trans-4-dienamides (II)—(IV) and piperine (I). Its mass spectrum showed  $M^+$  383 with intense fragment ion peaks at  $m/e$  161 and 248. On the basis of these facts we propose the structure (V), the peak at  $m/e$  161 being probably due to the fragment (z). Other

<sup>5</sup> R. Haensel, Ch. Leukert, and G. Schulz, *Z. Naturforsch.*, 1966, **621**, 530 (*Chem. Abs.*, 1966, **65**, 14,101d); R. Haensel and A. Pelter, *Arch. Pharm.*, 1969, **302**, 940; R. Haensel and D. Zander, *ibid.*, 1961, **294**, 699.

<sup>6</sup> L. Crombie, *J. Chem. Soc.*, 1955, 999, 1009.

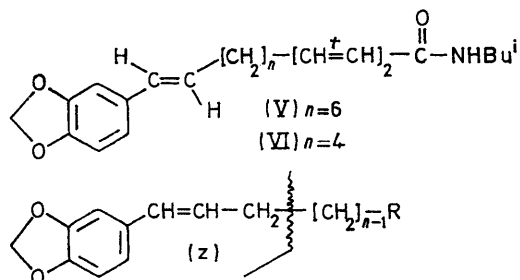
<sup>7</sup> T. Cairns, 'Spectroscopic Problems in Organic Chemistry,' vol. I, Heyden and Sons Ltd., London, 1964, Problem 37.

<sup>8</sup> A. J. Birch, P. L. Macdonald, and A. Pelter, *J. Chem. Soc. (C)*, 1967, 1968.

<sup>9</sup> A. Pelter, *J. Chem. Soc. (C)*, 1967, 1376.

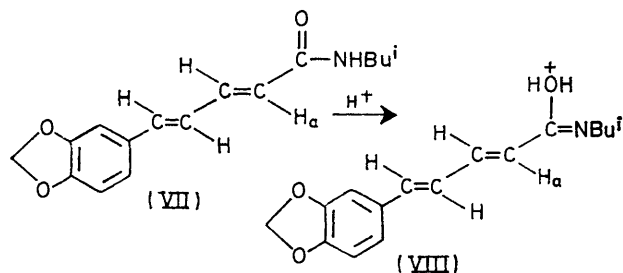
<sup>10</sup> L. H. Briggs, L. D. Colebrook, H. M. Fales, and W. C. Wildman, *Analyt. Chem.*, 1957, **29**, 904.

characteristic peaks were at  $m/e$  135, 131, and 103. An intense u.v. absorption at 260 nm is ascribable to the presence of the styryl chromophore together with the conjugated dienamide system, and the spectra were in



agreement with observations<sup>11,12</sup> for similar systems. The mass spectrum of an impure sample of guineensine showed a peak at  $m/e$  355 due to an impurity which was probably the lower homologue (VI).

Fraction (iv) was piperlonguminine (VII),<sup>4,13,14</sup> spectroscopically identical with a sample partially synthesised from trichostachine. We were forced to make this comparison because the <sup>1</sup>H n.m.r. spectrum of our sample of piperlonguminine was significantly different from the spectrum reported:<sup>14</sup> the signal due to one of the vinyl protons [probably H<sub>a</sub> in (VII)] in our spectrum was at  $\tau$  4.05 (1H, d,  $J$  14.5 Hz) instead of  $\tau$  3.75, and the signal



due to the methylene protons of the isobutyl group in our spectrum was a triplet ( $J$  6.5 Hz) around  $\tau$  6.85 instead of a multiplet (crude doublet,  $J$  8 Hz) around  $\tau$  6.5. The differences are considered to have arisen in the previous work from enolisation of (VII) to give (VIII), and a rapid proton exchange between the acidic medium and the protonated amide group. Indeed on acidifying a solution of our sample of piperlonguminine the n.m.r. spectrum changed to the form published. A similar observation was made with the <sup>1</sup>H n.m.r. spectrum of *N*-isobutylcinnamamide, the absorption of the butyl methylene group changing from a triplet to a crude doublet on acidification. It has been shown<sup>15</sup> that amides are protonated on oxygen rather than on nitrogen.

Fraction (v) was piperine, having spectral data identical with those reported.<sup>7</sup>

<sup>11</sup> J. Press and R. Brun, *Helv. Chim. Acta*, 1954, **37**, 190.  
<sup>12</sup> R. Grewe, W. Freist, H. Neumann, and S. Kersten, *Chem. Ber.*, 1970, **103**, 3752.  
<sup>13</sup> A. Chatterjee and C. P. Dutta, *Tetrahedron Letters*, 1966, 1797.

Fraction (vi) was trichostachine (I; pyrrolidide instead of piperidide),<sup>16,17</sup> showing spectral properties in agreement with its structure.

The structures of guineensine (V) and its lower homologue (VI) are derivable by the biogenetic pathway suggested<sup>12</sup> for similar compounds. Hitherto extracts of the genus *Piper* have been shown to contain amides derived from piperidine and isobutylamine,<sup>3,4,13,14</sup> or piperidine and pyrrolidine,<sup>12</sup> or pyrrolidine and isobutylamine.<sup>18</sup> This, to our knowledge, is the first instance of amides of the three amines co-occurring in the same fruit.

#### EXPERIMENTAL

Petroleum refers to the fraction of b.p. 60–80°. M.p.s were taken with a Kofler hot-stage apparatus. <sup>1</sup>H N.m.r. spectra were run for solutions in deuteriochloroform with Me<sub>4</sub>Si as internal standard, with a Varian A56/60 instrument. Except where otherwise stated mass spectra were obtained with a Perkin-Elmer-Hitachi RMU-6E instrument. Activated alumina refers to Laporte type H.

The fresh fruits of *P. guineense* were collected from Ojo (a village near Ibadan) in December–January.

**Extraction.**—The fruits were dried in the sun for 8 h and then at 60° for 36 h. They were then crushed and extracted (Soxhlet) with petroleum, chloroform, and methanol in that order. As the petroleum and chloroform extracts were qualitatively the same (t.l.c.), the initial extraction with petroleum was omitted in subsequent experiments. In a typical experiment, fresh fruits (750 g) yielded a thick brown oil (total 30 g) from the petroleum and chloroform extracts and a gum (20 g; wet with water) from the methanolic extract.

**Chromatography of Extract.**—The chloroform extract (27 g) from fruits not previously extracted with petroleum was chromatographed on a column of activated alumina (690 g). The eluates were analysed by t.l.c. (see Table).

Eluant	Products
Petroleum	Hydrocarbon oils
Petroleum-benzene (1 : 1)	Reddish gum (3 g) (M1)
Petroleum-benzene (1 : 3)	Reddish gum (4.7 g) (M2)
Benzene	Reddish gum (1 g) (M3)
Benzene	Reddish gum (1.3 g) (M4)
Benzene	Reddish gum (0.9 g) (M5)
Petroleum-benzene-chloroform (1 : 3 : 4)	{ Semisolid (2 g) (M6)
Chloroform	{ Semisolid (4 g) (M7)
	Gum (0.4 g); not investigated

*Sesamin*, *N*-Isobutylhexadeca-trans-2,trans-4-dieneamide (II), *N*-Isobutyloctadeca-trans-2,trans-4-dieneamide (III), and *N*-Isobutyleicosa-trans-2,trans-4-dieneamide (IV).—The mixture M1 on trituration with petroleum gave crystals of sesamin (20 mg), m.p. 121–123° (from ethanol) (lit.,<sup>10</sup> 123–124°),  $M^+$  352, spectra in agreement with published data.<sup>5,8–10</sup>

The petroleum mother-liquor gave a gum, part of which

<sup>14</sup> A. Chatterjee and C. P. Dutta, *Tetrahedron*, 1967, **23**, 1769; C. K. Atal and S. S. Banga, *Current. Sci. (India)*, 1963, **32**, 354 (*Chem. Abs.*, 1963, **59**, 15,329g).

<sup>15</sup> C. R. Smith, *Canad. J. Chem.*, 1972, **50**, 771.

<sup>16</sup> T. Szeki, *Math. naturw, Anz. ungar. Akad. Wiss.*, 1936, **54**, 807.

<sup>17</sup> J. Singh, K. L. Dhar, and C. K. Atal, *Tetrahedron Letters*, 1969, 4975.

<sup>18</sup> C. K. Atal, D. N. Moza, and A. Pelter, *Tetrahedron Letters*, 1968, 1397.

(1 g) was chromatographed on alumina. Benzene-petroleum (3 : 7) eluted a mixture of amides (0.4 g) as a semi-solid. Three recrystallisations from petroleum gave crystals, m.p. 83—86°;  $\nu_{\max}$ . (Nujol) 3300, 1655, 1630, 1615, and 1550 (unsaturated amide), 998 (*trans*-CH=CH),<sup>7</sup> and 720  $\text{cm}^{-1}$  ( $[\text{CH}_2]_n$ );  $\lambda_{\max}$ . (EtOH) 260 nm ( $\epsilon$  26,600 based on average mol. wt. 330);  $\tau$  ( $\text{CDCl}_3$ ) 9.1 (9H, d,  $J$  5.5 Hz), 8.75 (s,  $[\text{CH}_2]_n$ ), 6.85 (2H, t,  $J$  6 Hz), 3.8—4.4 (3H, m), and 2.9 (1H, m);  $m/e$  307, 335, and 363 ( $M^+$ ; intensities 335 > 307 >> 363) and 235 and 263 (Found: C, 78.65; H, 12.6; N, 4.25. Calc. for  $\text{C}_{20}\text{H}_{39}\text{NO}$ : C, 77.6; H, 12.7; N, 4.55. Calc. for  $\text{C}_{22}\text{H}_{43}\text{NO}$ : C, 78.25; H, 12.85; N, 4.15. Calc. for  $\text{C}_{24}\text{H}_{47}\text{NO}$ : C, 78.85; H, 12.95; N, 3.85%).

**Hydrogenation of the mixture of dienamides.** The mixture of dienamides (108 mg) and platinum oxide (18 mg) in methanol (100 ml) was shaken with hydrogen at atmospheric pressure until absorption stopped (1 h). Removal of solvent and catalyst left a crystalline residue (105 mg), m.p. 71—73°, after two recrystallisations from petroleum (*cf.* *N*-isobutylhexadecanamide, m.p. 66—68°; *N*-isobutyloctadecanamide, m.p. 73—75°; 1 : 1 mixture of *N*-isobutylhexadecan- and octadecan-amides, m.p. 68—70°);  $\nu_{\max}$ . (Nujol) 3300, 1625, and 1515 (CONHR) and 715  $\text{cm}^{-1}$  ( $[\text{CH}_2]_n$ ). G.l.c.—mass spectral analysis showed the presence of three amides [column 3% OV 225 on GasChrom Q (100—120 mesh); 150—230°]: (i)  $m/e$  311 ( $M^+$ ), 268 ( $M^+ - 43$ ), 256, and 239 (spectrum identical with that of *N*-isobutylhexadecanamide); (ii)  $m/e$  339 ( $M^+$ ), 296 ( $M^+ - 43$ ), 284, and 267 (spectrum identical with that of *N*-isobutyloctadecanamide); (iii)  $m/e$  367 ( $M^+$ ), 324 ( $M^+ - 43$ ), 312, and 295.

**Conversion of the mixture of hydrogenated amides into the corresponding mixture of methyl esters.** The mixture of saturated amides (30 mg) in methanolic potassium hydroxide (20%; 15 ml) was heated at reflux for 15 h. The methanol was removed under reduced pressure. The semi-solid residue was shaken vigorously with water and petroleum until it all had dissolved. The aqueous layer was acidified (dil. HCl) and extracted with ether. The dried ( $\text{MgSO}_4$ ) extract was treated with an excess of ethereal diazomethane. Removal of solvent left a solid which g.l.c.—mass spectral analysis showed to be a mixture of three methyl esters: (i)  $m/e$  270 ( $M^+$ ), 227 ( $M^+ - 43$ ), and 150, corresponding to methyl hexadecanoate; (ii)  $m/e$  298 ( $M^+$ ), 255 ( $M^+ - 43$ ), and 150, corresponding to methyl octadecanoate; and (iii)  $m/e$  326 ( $M^+$ ), 283 ( $M^+ - 43$ ), and 150, corresponding to methyl eicosanoate.

**Guineensine.**—The mixture M2 (800 mg) was rechromatographed on activated alumina. Elution with benzene-petroleum (3 : 7) gave first a solid mixture of two sterols (i.r.), m.p. 138°,  $M^+$  424 and 426; then crude guineensine. Three recrystallisations from benzene-petroleum gave the pure *N*-isobutyl-13-(3,4-methylenedioxyphenyl)trideca-2,4,12-trienamide, m.p. 113—115°;  $\nu_{\max}$ . (Nujol) 3300, 1655, 1630, 1605, and 1545 (unsaturated amide), 1000 (*trans*-CH=CH), 965 (styryl C=C), and 925  $\text{cm}^{-1}$  (O-CH<sub>2</sub>-O);  $\lambda_{\max}$ . (EtOH) 261 ( $\epsilon$  53,600) and 302 nm (6500);  $\tau$  ( $\text{CDCl}_3$ ) 2.9 (1H, m), 3.1—3.25 (3H, m), 3.8—4.35 [7H, m, including 4.1 (2H, s, O-CH<sub>2</sub>-O), 6.85 (2H, t,  $J$  6.5 Hz), 8.6 (s,  $[\text{CH}_2]_n$ ), and 9.05 (6H, d,  $J$  6 Hz);  $m/e$  383 ( $M^+$ ), 248, 161, 135, 131, and 103 (Found: C, 75.65; H, 8.7; N, 3.6%;  $M^+$ , 383.2465. Calc.

for  $\text{C}_{24}\text{H}_{33}\text{NO}_3$ : C, 75.15; H, 8.65; N, 3.65%;  $M$ , 383.2460). An impure sample of guineensine showed  $m/e$  355 ( $M^+$ ,  $\text{C}_{22}\text{H}_{29}\text{NO}_3$ ) and 220.

**Piperine (I).**—The mixture M4 crystallised from benzene to give piperine (1 g), m.p. 128—130° (from ethanol) (lit.,<sup>19</sup> 128—129.5°), spectral properties identical with published data.<sup>7</sup>

**Piperlonguminine (VII).**—The mixture M5 on rechromatography on activated alumina and elution with benzene-petroleum (3 : 7) gave piperlonguminine (0.2 g), m.p. and mixed m.p. 162—164° (from benzene-petroleum) (lit.,<sup>4,13,14</sup> 161—163, 166—168°);  $\nu_{\max}$ . (Nujol) 3400, 3150, 1645, 1610, 1550, 1225, 1020, 990, 925, 850, and 802  $\text{cm}^{-1}$ ;  $\lambda_{\max}$ . (EtOH) 340 nm ( $\epsilon$  27,300), 305 (17,440), 295 (15,170), 255 ( $\epsilon$  10,230), and 242 nm (9480);  $\tau$  ( $\text{CDCl}_3$ ) 2.7 (1H, m), 3—3.4 (5H), 4.05 (1H, d,  $J$  14.5 Hz), 4.1 (2H, s), 6.85 (2H, t,  $J$  6.5 Hz), 8.2 (1H, m), and 9.1 (6H, d,  $J$  6 Hz);  $\tau$  ( $\text{CDCl}_3$ -HCl) 2.55 (1H, m), 3.75 (1H, d,  $J$  14.5 Hz), and 6.7 (2H, crude d,  $J$  8 Hz);  $m/e$  273 ( $M^+$ ) 201 (base peak), and 173.

**Trichostachine (I; pyrrolidine instead of piperidide).**—The mixture M7 crystallised from benzene-petroleum to give trichostachine, m.p. 143—145° (lit.,<sup>16,17</sup> 144, 142—143°), i.r., u.v., and n.m.r. very similar to those of piperine,  $m/e$  271 ( $M^+$ ) and 201.

**Conversion of Trichostachine into Piperlonguminine (VII) (cf. ref. 14).**—Trichostachine (1.1 g) in methanolic potassium hydroxide (20%; 50 ml) was heated at reflux protected from light under nitrogen for 14 h. The precipitate obtained on cooling (ice-bath) was collected and washed with cold methanol. Treatment with hydrochloric acid gave piperic acid (0.2 g). The methanolic filtrate was evaporated and the residue was shaken vigorously with water and ether until it dissolved. The ether layer gave the starting material (0.3 g; m.p. 142—144°). The aqueous layer was acidified with 6*N*-hydrochloric acid and extracted with chloroform. The extract was dried ( $\text{MgSO}_4$ ), filtered, and distilled to leave more piperic acid (0.4 g), m.p. 215—218° (from ethanol) (lit.,<sup>20</sup> 215°). The piperic acid was treated in the cold (ice-bath) with thionyl chloride (2 ml) and the mixture was kept overnight at room temperature. The excess of thionyl chloride was removed by co-distillation with dry benzene under reduced pressure. The residue was taken up in dry benzene (40 ml) and added dropwise to a cooled solution (ice-bath) of an excess of isobutylamine in pyridine (3 ml) and dry benzene (10 ml). The mixture was left for 7 h at room temperature, then diluted with benzene (60 ml), washed with dilute hydrochloric acid, sodium hydrogen carbonate solution, and water, dried ( $\text{MgSO}_4$ ), filtered, and evaporated. The product was chromatographed on alumina to give piperlonguminine (150 mg after recrystallisation from benzene-petroleum), m.p. and mixed m.p. (with the natural product) 162—164° (Found: C, 70.5; H, 7.0; N, 4.7. Calc. for  $\text{C}_{16}\text{H}_{19}\text{NO}_3$ : C, 70.3; H, 7.0; N, 5.1%), i.r., u.v., n.m.r., and mass spectra identical with those of the natural material.

**Isobutylamides of Cinnamic, Hexadecanoic, and Octadecanoic Acids.**—These were made from the appropriate acids by the procedure described for conversion of piperic acid into piperlonguminine. *N*-Isobutylcinnamamide had m.p. 112—114° (lit.,<sup>21</sup> 114°). *N*-Isobutylhexadecanamide had m.p. 68—70° (from petroleum) (Found: C, 77.2; H, 13.2; N, 4.3.  $\text{C}_{20}\text{H}_{41}\text{NO}$  requires C, 77.1; H, 13.3; N, 4.5%);  $\nu_{\max}$ . (Nujol)

<sup>19</sup> W. Karrer, 'Konstitution und Vorkommender organischen Pflanzenstoffe,' Birkhauser, Basel, 1956, p. 406.

<sup>20</sup> 'Dictionary of Organic Compounds,' Eyre and Spottiswoode, London, 1965, vol. 4, p. 2193.

<sup>21</sup> P. C. Mitter and S. C. Ray, *J. Indian Chem. Soc.*, 1937, **14**, 421 (*Chem. Abs.*, 1938, **32**, 491<sup>2</sup>).

3300, 1625, 1535, and 715  $\text{cm}^{-1}$ ;  $m/e$  311 ( $M^+$ ), 268, 256, and 239. *N-Isobutyloctadecanamide* had m.p. 74–76° (from petroleum) (Found: C, 77.6; H, 13.5; N, 4.1.  $\text{C}_{22}\text{H}_{45}\text{NO}$  requires C, 77.8; H, 13.4; N, 4.1%);  $\nu_{\text{max}}$  (Nujol) 3300, 1625, 1535, and 715  $\text{cm}^{-1}$ ;  $m/e$  339 ( $M^+$ ), 296, 284, and 267.

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