Isolation and Identification of Amides from *Piper callosum*. Synthesis of Pipercallosine and Pipercallosidine

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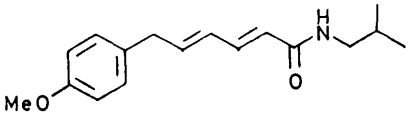
Extraction of the roots of the shrub *Piper callosum* with cyclohexane afforded piperovatine (1) [*N*-isobutyl-6-(*p*-methoxyphenyl)sorbamide] and two new amides, the isobutylamides of (2*E*,4*E*)-9-(3,4-methylenedioxyphenyl)-nona-2,4-dienoic acid and (*E*)-7-(3,4-methylenedioxyphenyl)hept-2-enoic acid, named pipercallosine (3) and pipercallosidine (14), respectively. The structures of the two new amides have been confirmed by synthesis.

Piper callosum Ruiz and Pavon (Piperaceae) is a shrub native to Bolivia, Brazil, Peru, and Colombia. It is used in indigenous medicine as an ingredient in preparations for alleviating pain such as toothache. The genus *Piper* is used in popular medicine in several parts of the world, and has been widely investigated chemically in recent years, many of the compounds isolated being amides of unsaturated arylalkanoic acids.[1-4] Among the compounds isolated from *Piper* species, at least two having local anaesthetic properties have been reported, namely jaborandine [5] from *Piper jaborandi* and piperovatine [6] from *Piper ovatum*. The latter compound has been characterized as *N*-isobutyl-6-(*p*-methoxyphenyl)sorbamide.[7]

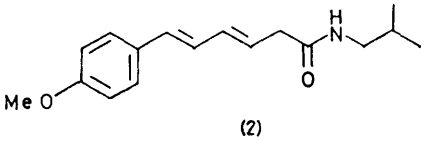
In order to isolate the anaesthetically active principle of *Piper callosum*, powdered roots of the shrub were extracted with cyclohexane. A mixture of compounds was obtained which was triturated with hexane. The hexane solution afforded a semi-crystalline precipitate which was chromatographed on silica to afford a crystalline compound, m.p. 120—121 °C, with local anaesthetic activity. This compound was shown to be identical with piperovatine (1). The hexane filtrate obtained after separation of the semi-crystalline precipitate was evaporated to afford a gum which was also chromatographed on silica. A second crystalline compound, m.p. 114—115 °C, was obtained which was lacking in anaesthetic properties. This compound was named pipercallosine. Certain fractions from the chromatographic eluate were shown (g.l.c.) to contain a third compound. These fractions were combined and chromatographed on alumina to afford a third crystalline compound, m.p. 80—82 °C, which was named pipercallosidine.

When chromatography of the cyclohexane extract of the powdered roots was carried out on alumina, piperovatine (1) was not present in the eluted fractions. Instead, a compound, m.p. 118—120 °C, isomeric with piperovatine, was obtained. The u.v. spectrum of this compound in cyclohexane [$\lambda_{max.}$ 296 nm (ε 32 500)] indicated that a hydrogen shift had occurred in piperovatine [$\lambda_{max.}$ (cyclohexane) 256.5 nm (ε 24 550)] yielding compound (2) in which there is conjugation of the diene system with the aromatic ring. A similar base-catalysed rearrangement has been reported for the isobutylamide of 5-(2-thienyl)penta-2,4-dienoic acid.[8]

High-resolution mass spectrometry indicated that the exact mass of the molecular ion of pipercallosine (*m/z* 329.2038) corresponds to that of the molecular formula $C_{20}H_{27}NO_3$. The neutral character of the compound suggested that it might be an amide and this was confirmed by its i.r. spectra in CCl_4 [maxima at 3 455 (free NH), 3 310 (H-bonded NH), and 1 674 cm^{-1} (amide I)] and KBr [maxima at 3 290 (H-bonded NH), 1 660 (amide I), and 1 550 cm^{-1} (amide II)]. The presence of



(1)



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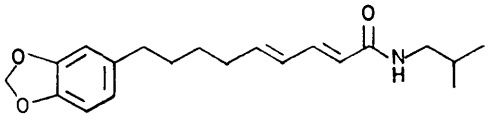
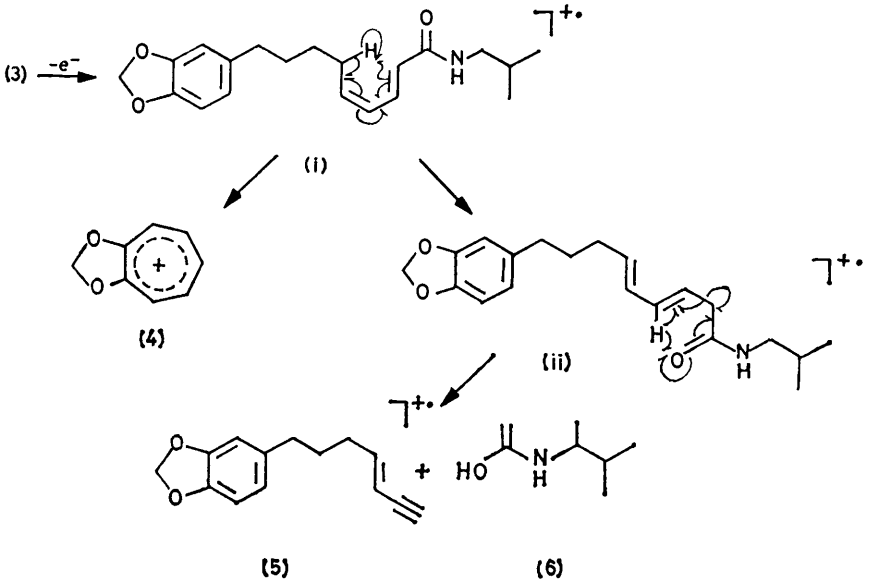


(3)

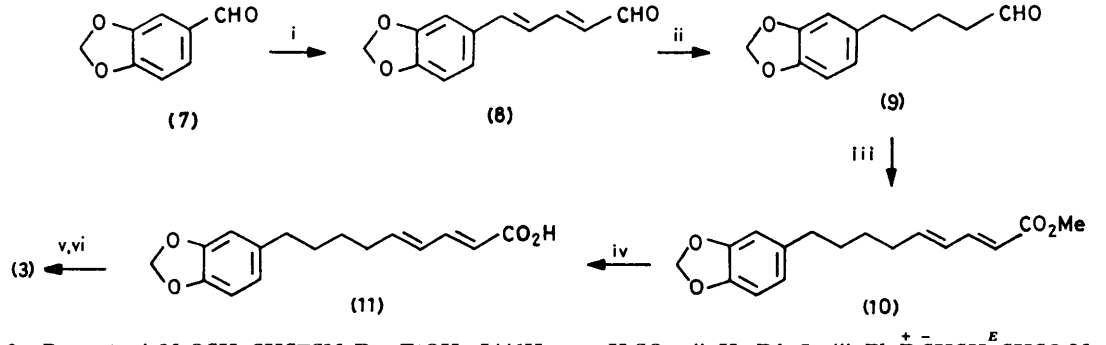
bands at 2 780, 1 255, 1 046, and 928 cm^{-1} indicated a methylenedioxy-group and that at 997 cm^{-1} suggested a diene system conjugated with the amide carbonyl group, the diene system probably having the *trans-trans* configuration (*cf.* ref. 9). The presence of the conjugated dieneamide system was supported by the u.v. spectrum [$\lambda_{max.}$ (cyclohexane): 252.5 nm (ε 31 000)]. The 60 MHz ^1H n.m.r. spectrum confirmed the presence of a methylenedioxy-group (2 H-singlet at δ 5.90) and indicated the presence of an isobutylamide structure (6 H-doublet at δ 0.91 and 2 H-triplet at δ 3.16). In addition, a 4 H-multiplet at δ 6.4—7.4 corresponded to 3 aromatic protons and an olefinic proton, and a 6 H-multiplet at δ 5.2—6.2, which contained the 2 H-singlet, indicated a further three olefinic protons and probably contained also an amide proton resonance. A 9 H-multiplet corresponding to the isobutyl methine proton and eight methylene protons was seen at δ 1.1—2.8. The mass spectrum showed intense fragment-ion peaks at *m/z* 214 and *m/z* 135 (base peak) due to ions with the composition $C_{14}H_{14}O_2$ and $C_8H_7O_2$, respectively, according to

exact mass determinations. On the basis of these data, structure (3) is proposed for pipercallosine, with some reservation as to the configuration of the double bonds. The peak at *m/z* 135 is ascribed to the methylenedioxytropylium ion (4),[10] while that at *m/z* 214 corresponds to the ion (5), which may arise from the molecular ion (i) *via* hydrogen migration to give ion (ii) followed by a McLafferty rearrangement with charge retention by the unsaturated chain (Scheme 1). The ion at *m/z* 115 indicates that there is some charge retention by the enol fragment (6). On hydrogenation, pipercallosine took up 2 mol equiv. of hydrogen as indicated by the mass spectrum of the hydrogenation product, which shows a molecular ion at *m/z* 333. The most intense fragment ion at *m/z* 115 results from a McLafferty rearrangement.



SCHEME 1

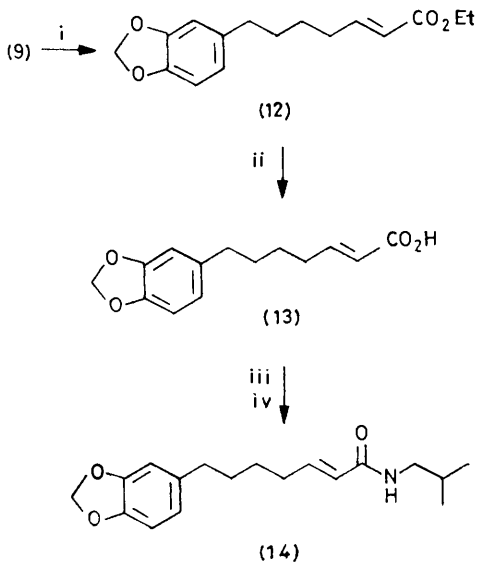
The proposed structure (3) for pipercallosine was confirmed by synthesis (Scheme 2). Thus, (2*E*,4*E*)-5-(3,4-methylenedioxyphenyl)penta-2,4-dienal (8) was synthesized in 64% yield from piperonal (7) and the Grignard reagent from 1-methoxybut-1-en-3-yne by analogy with Marshall and Whiting's synthesis of 5-(*p*-methoxyphenyl)penta-2,4-dienal.[11] Hydrogenation of compound (8) over palladium–charcoal in ethyl acetate solution afforded the aldehyde (9) which polymerised with time to give a white solid; this had a mass spectrum similar to that of the aldehyde, except for a weak peak at *m/z* 618 which indicates that it is a cyclic trimer. Freshly distilled aldehyde (9) was condensed with the ylide of (*E*)-(3-methoxycarbonylprop-2-enyl)triphenylphosphonium bromide [12] giving methyl 9-(3,4-methylenedioxyphenyl)nona-2,4-dienoate as a *ca.* 2 : 1 mixture of the (2*E*,4*E*)- [compound (10)] and (2*E*,4*Z*)-isomers together with a little ethyl ester formed by trans esterification as indicated by g.l.c.–mass spectrometry. The mixture was dissolved in ether–hexane and a couple of iodine crystals were added. On exposure to sunlight, most of the (2*E*,4*Z*)-isomer isomerized to afford an 8 : 1 mixture of the two aforementioned methyl esters (*cf.* ref. 13). The crude (2*E*,4*E*)-isomer (10) was converted into the acid (11) by alkaline hydrolysis. A sample of the recrystallised acid (11) was methylated with diazomethane and subjected to g.l.c. A peak (>98%)



SCHEME 2 *Reagents:* i, MeOCH=CHC≡CMgBr; EtOH; $LiAlH_4$; aq. H_2SO_4; ii, H_2–Pd–C; iii, $Ph_3\overset{+}{P}\text{-}\overset{-}{C}HCH\overset{E}{=}CHCO_2Me$–EtOH; I_2, *hν*; iv, KOH–MeOH; aq. HCl; v, $(COCl)_2$–Et_2O; vi, Bu^tNH_2–Et_2O

corresponding to the main component of the crude ester (10), *i.e.* the (2*E*,4*E*)-isomer, was observed. The acid (11) reacted with oxalyl chloride in ether to give the acid chloride which was treated *in situ* with isobutylamine in ether giving the amide (3), identical in all respects with natural pipercallosine. Hydrogenation of the synthetic amide gave *N*-isobutyl-9-(3,4-methylenedioxyphenyl-nonanamide which had a mass spectrum identical with that of hydrogenated pipercallosine.

The i.r. spectra of pipercallosidine in KBr and CCl_4 indicated the presence of a secondary amide group [maxima at 3 300 (H-bonded NH), 1 668 (amide I), and 1 554 cm^{-1} (amide II) in KBr and at 3 450 (free NH), 3 300 (H-bonded NH), and 1 685 cm^{-1} (amide I) in CCl_4]. Furthermore, peaks at 1 631 and 978 cm^{-1} indicated the



SCHEME 3 *Reagents:* i, $(EtO)_2P(O)CH_2CO_2Et$–NaH–THF; ii, KOH–MeOH; aq. HCl; iii, $(COCl)_2$–C_6H_6; iv, Bu^iNH_2–C_6H_6

presence of a conjugated *trans*-double bond. An exact mass determination of the molecular ion of pipercalosidine at *m/z* 303.1821 suggested the molecular formula $C_{18}H_{25}NO_3$. The low-resolution mass spectrum gave a base peak at *m/z* 135 corresponding to the methylenedioxytropylium ion (4). Peaks at *m/z* 115 and 188 correspond to ions deriving from a McLafferty rearrangement and indicate the presence of an isobutylamide group. On the basis of these data pipercallosidine was assigned structure (14). The 100 MHz ^1H n.m.r. spectrum was in accord with this structure. Thus, the presence of the isobutylamide group was indicated by a 6 H-doublet at δ 0.93 and a 2 H-triplet at δ 3.15. The methylenedioxy-protons gave a singlet at δ 5.91, and the benzylic protons were centred at δ 2.54. An olefinic proton at δ 5.73 appeared as a double triplet with the larger coupling constant of 14.5 Hz indicating the *trans*-configuration. 5 H- and 2 H-Multiplets at δ 1.3—1.9 and 2.0—2.3 corresponded to the isobutyl methine proton and six methylene protons. A 4 H-multiplet at δ 6.5—7.1 contained signals from three aromatic protons and an olefinic proton.

In order to verify the proposed structure for pipercallosidine, a synthesis was carried out (Scheme 3). Thus 5-(3,4-methylenedioxyphenyl)pentanal (9) and ethyl (diethoxyphosphoryl)acetate reacted together in the presence of sodium hydride in tetrahydrofuran (THF) solution to give a mixture of isomers in which the *trans*-product, ethyl (2*E*)-7-(3,4-methylenedioxyphenyl)-hept-2-enoate (12), was the dominant product according to g.l.c.–mass spectrometry (which indicated an isomeric ratio of 96 : 4) and ^1H n.m.r. (which showed an olefinic coupling of 15 Hz for the doublet at δ 5.77). Alkaline hydrolysis of this ester afforded the free acid (13) which was converted into the amide (14) *via* the acid chloride by reaction with oxalyl chloride in benzene followed by treatment with isobutylamine in the same solvent. The product was identical in all respects with natural pipercallosidine.

EXPERIMENTAL

M.p.s were determined with a Büchi–Tottoli apparatus. I.r. spectra were recorded on Perkin-Elmer 720 or 457 instruments and u.v. spectra with a Zeiss DMR 21 spectrophotometer. ^1H N.m.r. spectra were obtained for solutions in deuteriochloroform with tetramethylsilane as internal reference at 60 MHz on Varian A 60 A or T 60 instruments or, if stated, at 100 MHz on a Varian XL 100 instrument. G.l.c. was carried out using a Perkin-Elmer 3920 gas chromatograph equipped with a flame-ionization detector with nitrogen as carrier gas at a flow rate of 30 ml min^{-1} and g.l.c.–mass spectrometry was performed with a Pye Unicam Series 104 gas chromatograph (helium as carrier gas, flow rate of 30 ml min^{-1}) coupled to an LKB 2091 mass spectrometer. Other low-resolution mass spectra were recorded with an LKB 9000 instrument using the probe inlet. Exact mass determinations were performed with an Atlas SM 1 instrument. T.l.c. was carried out on Merck silica gel GF_{254} using isopropyl ether as developing solvent with detection by iodine vapour.

Isolation of Piperovatine (1), *Pipercallosine* (3), *and Pipercallosidine* (14).—Air-dried roots of *P. callosum* plants obtained from Bolivia were finely pulverized and powdered material (250 g) was extracted (Soxhlet) with cyclohexane for 24 h. Removal of the solvent gave a residue (8 g) which was triturated with several portions of boiling hexane. On cooling, the pooled hexane solutions deposited a semi-crystalline precipitate (A) (0.48 g) which was filtered off. The filtrate was evaporated to dryness to afford a gum (B) (4.51 g). The precipitate (A) was chromatographed on silica gel (Merck 0.05—0.20 mm) (25 g) using isopropyl ether as eluant. Fractions were examined by t.l.c. and those containing the major component (R_F 0.17) were combined and evaporated to dryness to afford a solid residue (0.25 g). Attempted recrystallisation from isopropyl ether or cyclohexane resulted in gel formation but, on slow cooling of an ethereal solution, crystals (0.16 g) were formed, m.p. 120.5—121.5 °C. The compound was shown to be identical with an authentic specimen of piperovatine [7] [*N*-isobutyl-6-(*p*-methoxyphenyl)hexa-2,4-dienamide] (m.p. 120—121 °C) by mixed m.p., ^1H n.m.r. and i.r. spectra, and t.l.c.

The gum (B) was chromatographed on silica gel (Merck

0.05—0.20 mm) (80 g) using gradient elution with benzene–isopropyl ether as eluant.

(a) Fractions were examined by t.l.c., and those containing a component with R_F 0.21 were combined and evaporated to afford a solid (0.15 g) which was recrystallised twice from cyclohexane and twice from aqueous ethanol to afford *pipercallosine* (3), m.p. 114—115 °C; $\lambda_{max.}$ (cyclohexane) 252.5 nm (ε 31 000); $\nu_{max.}$ (KBr) 3 290, 1 660, 1 630, 1 618, and 1 550 (unsaturated secondary amide), 997 (*trans* CH=CH), 2 780, 1 255, 1 046, and 928 cm^{-1} OCH_2O); $\nu_{max.}$ (CCl_4) 3 455, 3 310, 1 674, 1 637, and 1 620 cm^{-1} (unsaturated secondary amide); δ 0.91 (6 H, d, *J* 6.5 Hz), 1.1—2.8 (9 H, m), 3.16 (2 H, t, *J* 6.5 Hz), 5.2—6.2 (4 H, m), 5.90 (2 H, s), and 6.4—7.4 (4 H, m); *m/z* 329 (M^+, 29), 257 (8), 229 (16), 214 (31), 194 (21), 180 (12), 166 (6), 161 (9), 154 (18), 152 (28), 148 (14), 141 (23), 135 (100), 131 (13), 128.5 (22), 115 (15), 105 (10), 91 (10), 81 (17), 79 (13), 77 (29), 66 (12), 57 (27), 55 (26), 51 (11), 41 (19), and 39 (10) [Found: M^+, 329.2038; also *m/z* 214.1021 and 135.0464. $C_{20}H_{27}NO_3$, $C_{14}H_{14}O_2$, and $C_8H_7O_2$ require *m/z* 329.1991 (*M*), 214.0994, and 135.0446, respectively]. A sample of pipercallosine (3) (2 mg) was hydrogenated for 1 h over Adams (platinum oxide) catalyst in ethanol. After filtration and removal of the solvent, the residue gave peaks at *m/z* 333 (M^+, 100), 290 (7), 261 (11), 198 (19), 170 (5), 142 (8), 135 (29), 128 (47), 115 (80), 74 (17), 60 (20), and 57 (7).

(b) Fractions containing a component with R_F 0.17 were combined and evaporated to give piperovatine (1) (0.06 g). Similar fractions from the chromatography of the semi-crystalline precipitate (A) as well as from that of the gum (B) were combined and investigated by g.l.c. (3% OV-1 on Gas Chrom Q, column length 2 m, temperature programming from 240—280 °C at 2 °C min^{-1}). In addition to piperovatine (1) and pipercallosine (3) (ratio of retention times 1 : 2.2), certain fractions contained a component (C) with a retention time of 1.2 relative to that of piperovatine. One fraction (20 mg), in which this was the main component, was chromatographed on Merck neutral alumina (activity grade III) (11 g) with gradient elution using benzene–isopropyl ether as eluant. Fractions were investigated by g.l.c. Those containing component (C) were combined and evaporated to dryness to give a solid residue (14 mg) which was recrystallised from cyclohexane to afford *pipercallosidine* (14), m.p. 80—82 °C; $\lambda_{max.}$ (cyclohexane) 294.5 (ε 2 820), 289 (3 910), 284.5 (4 000), and 232sh nm (8 630); $\nu_{max.}$ (KBr) 3 300, 1 668, 1 631, and 1 554 (unsaturated secondary amide), 978 (*trans* CH=CH), 2 780, 1 256, 1 044, and 925 cm^{-1} (OCH_2O); $\nu_{max.}$ (CCl_4) 3 450, 3 300, 1 685, and 1 632 cm^{-1} (unsaturated secondary amide); δ (100 MHz) 0.93 (6 H, d, *J* 6.5 Hz), 1.3—1.9 (5 H, m), 2.0—2.3 (2 H, m), 2.54 (2 H, crude t, *J* 7 Hz), 3.15 (2 H, t, *J* 6.5 Hz), 5.41br. (1 H, s), 5.73 (1 H, dt, *J* 14.5 and 1.2 Hz), 5.91 (2 H, s), and 6.5—7.1 (4 H, m); *m/z* 303 (M^+, 15), 203 (12), 188 (12), 168 (14), 154 (33), 148 (9), 141 (7), 135 (100), 115.5 (13), 115 (11), 108 (9), 81 (10), 77 (20), 60 (9), 57 (22), and 41 (15) (Found: M^+, 303.1821. $C_{18}H_{25}NO_3$ requires *M*, 303.1824).

Isolation of N-*Isobutyl*-6-(p-*methoxyphenyl*)*hexa*-3,5-*dienamide* (2), *the Rearrangement Product of Piperovatine*.—The residue (2.5 g) from a cyclohexane extract of the pulverized roots of *P. callosum* was chromatographed on neutral alumina (Merck, activity I) (125 g) with gradient elution using benzene–isopropyl ether–chloroform as eluant. Fractions were investigated by t.l.c. and those containing a component with R_F 0.08 (not present in the original extract) were combined and evaporated to afford a solid residue (0.44 g) which was recrystallised twice from hexane and once from toluene to give the *product*, m.p. 118—120 °C; $\lambda_{max.}$ (cyclohexane) 296 nm (ε 32 500); $\nu_{max.}$ (KBr) 3 230, 1 640, 1 605, and 1 550 (secondary amide), 990 (*trans* CH=CH), 1 236 (aryl ether), and 833 cm^{-1} (*para*-disubstituted aryl); δ 0.88 (6 H, d, *J* 6 Hz), 1.4—2.2 (1 H, m), 2.95—3.35 (4 H, m), 3.80 (3 H, s), 5.5—6.8 (5 H, m), and 7.09 (4 H, A_2B_2 pattern); *m/z* 273 (M^+, 52), 174 (45), 173 (100), 159 (34), 158 (26), 143 (10), 128 (11), 115 (16), 91 (6), 77 (5), 57 (36), 41 (19), and 39 (7) (Found: M^+, 273.1733. $C_{17}H_{33}NO_2$ requires *M*, 273.1729).

(2E,4E)-5-(3,4-*Methylenedioxyphenyl*)*penta*-2,4-*dienal* (8).—This compound was prepared by condensing the ethylmagnesium salt of 1-methoxybut-1-en-3-yne (14.6 g) with piperonal (20.0 g), followed by reduction of the hydrolysed complex with lithium aluminium hydride and hydrolysis with mineral acid. The neutral fraction was recrystallised from ethyl acetate–ether to give the aldehyde (8) as a red, crystalline product (17.2 g, 64.0%), m.p. 107—109 °C, raised to 108—109.5 °C on recrystallisation (lit.,[14] 89—90 °C) (Found: C, 71.2; H, 4.9. Calc. for $C_{12}H_{10}O_3$: C, 71.3; H 5.0%); $\lambda_{max.}$ ($CHCl_3$) 250.5, 269, 316.5, and 360 nm (ε 10 950, 11 170, 10 890, and 25 530); $\nu_{max.}$ (KBr) 1 674, 1 663, and 1 616 (unsaturated aldehyde), 985 (*trans* CH=CH), 2 798, 1 258, 1 040, and 930 cm^{-1} (OCH_2O); δ 5.99 (2 H, s), 6.20 (1 H, dd, *J* 15 and 8 Hz), 6.7—7.5 (6 H, m), and 9.54 (1 H, d, *J* 8 Hz); *m/z* 202 (M^+, 100), 173 (36), 144 (22), 143 (24), 116 (48), 115 (99), 89 (20), 63 (28), 51 (18), and 39 (22).

5-(3,4-*Methylenedioxyphenyl*)*pentanal* (9).—Compound (8) (10.11 g) was hydrogenated in ethyl acetate (200 ml) in the presence of 5% palladium–charcoal (0.75 g) at normal temperature and pressure. 2 Mol equiv. of hydrogen were taken up within 1.5 h. The catalyst was filtered off and the filtrate was evaporated to leave an oil (10.28 g) which was distilled giving the *aldehyde* (9) (9.22 g), b.p. 112—114 °C at 0.09 mmHg; n_D^{25} 1.5289 (Found: C, 69.6; H, 6.9. $C_{12}H_{14}O_3$ requires C, 69.9; H, 6.8%); $\nu_{max.}$ (film) 1 733 cm^{-1} (aldehyde); δ 1.2—2.0 (4 H, m), 2.05—2.9 (4 H, m), 5.88 (2 H, s), 6.45—6.85 (3 H, m), and 9.75 (1 H, t, *J* 3.0 Hz); *m/z* 206 (M^+, 25), 178 (3), 135 (100), 105 (6), 91 (5), 77 (18), 51 (11), and 39 (5). The 2,4-*dinitrophenylhydrazone* had m.p. 105.5—107 °C (ethanol) (Found: C, 55.9; H, 4.7; N, 14.3. $C_{18}H_{18}N_4O_6$ requires C, 56.0; H, 4.7; N, 14.5%).

On being kept at room temperature for a few days, a portion of the aldehyde polymerized to a white solid, m.p. 87.5—89.5 °C (cyclohexane). An i.r. spectrum showed no carbonyl absorption and the mass spectrum was similar to that of the aldehyde except for the presence of a very weak peak at *m/z* 618 which corresponds to that of the molecular ion of the *cyclic trimer*; δ($CDCl_3$) 1.2—2.0 (6*n* H, m), 2.3—2.8 (2*n* H, m), 4.80 (*n* H, crude t, *J* 5 Hz), 5.86 (2*n* H, s), and 6.45—6.85 (3*n* H, m) [Found: C, 69.9; H, 6.7. $(C_{12}H_{14}O_3)_n$ requires C, 69.9; H, 6.8%].

Methyl (2E,4E)-9-(3,4-*Methylenedioxyphenyl*)*nona*-2,4-*dienoate* (10).—To a solution of sodium (0.38 g, 0.0165 g-atom) in absolute ethanol (50 ml) under argon was added portionwise (*E*)-(3-methoxycarbonylprop-2-enyl)triphenylphosphonium bromide[12] (7.94 g, 0.0180 mol). After stirring of the orange-yellow suspension of the ylide for 1 h at room temperature, freshly distilled aldehyde (9) (3.09 g, 0.0150 mol) was added and the resulting red-brown solution was stirred at room temperature for 19 h. After removal of the solvent, the residue was stirred with ether and the mixture filtered. The filtrate was evaporated to dryness and the residue (5.31 g) was chromatographed on 210 g silica gel (Merck, 0.063—0.200 mm) (210 g) using gradient elution with ben-

zene–ethyl acetate as eluant. Fractions were investigated by t.l.c. and those containing components with R_F 0.54, 0.51, and 0.43 were combined and evaporated to dryness giving an oil (2.41 g). G.l.c. (3% OV 17 on a Gas Chrom Q column with temperature programming from 240—280 °C at 2 °C min^{-1}) showed three main peaks (with relative retention times 1 : 1.13 : 1.29) in the proportions 32 : 58 : 10. G.l.c.–mass spectrometry showed that the first and second peaks contained components giving a molecular-ion peak at *m/z* 288, their mass spectra being identical, and that the third peak contained a component with a molecular-ion peak at *m/z* 302 (ethyl ester). The oil (2.22 g) was dissolved in hexane–ether (500 ml; 3 : 2 v/v) and a couple of iodine crystals were added to the solution which was exposed to sunlight for 15 min. After washing with aqueous sodium thiosulphate and water, the solution was dried (Na_2SO_4) and evaporated to dryness to give an oil (2.2 g). G.l.c. showed the same three peaks as previously, but in the proportions 1 : 8 : 1; $\nu_{max.}$ (CCl_4) 1 720, 1 642, and 1 615 (unsaturated ester), 995 (*trans* CH=CH), 2 780, 1 240, 1 040, and 940 cm^{-1} (OCH_2O); δ 1.1—1.9 (4 H, m), 1.9—2.7 (4 H, m), 3.59 (3 H, s) 5.63 (1 H, d, *J* 15 Hz), 5.79 (2 H, s), 5.8—6.2 (2 H, m), and 6.3—7.4 (4 H, m) [peaks at δ 1.18 (t, *J* 7 Hz) and 4.07 (q, *J* 7 Hz) correspond to the presence of the ethyl ester as an impurity]; the main component showed *m/z* 288 (M^+, 23), 257 (5), 229 (7), 206 (10), 161 (15), 148 (16), 135 (100), 131 (27), 122 (8), 105 (9), 91 (7), 77 (26), 51 (11), and 39 (7).

(2E,4E)-9-(3,4-*Methylenedioxyphenyl*)*nona*-2,4-*dienoic Acid* (11).—To a solution of the crude methyl ester (10) (*ca.* 80% pure) (2.0 g) in 90% aqueous methanol (55 ml) was added 10% methanolic potassium hydroxide (10 ml). The mixture was stirred at room temperature under nitrogen for 22 h. After removal of most of the methanol under reduced pressure, water was added and the mixture was washed with ether. The aqueous phase was acidified with 2M hydrochloric acid and extracted with ether. The extracts were dried (Na_2SO_4) and evaporated to dryness to afford a crystalline residue (1.26 g) which was recrystallised twice from cyclohexane giving the *acid* (11) (0.65 g), m.p. 118—121 °C. An analytical sample, m.p. 120—121.5 °C, was obtained by further recrystallisation from aqueous ethanol (Found: C, 69.9; H, 6.6. $C_{16}H_{18}O_4$ requires C, 70.0; H, 6.6%); $\lambda_{max.}$ (95% EtOH) 254.5 nm (ε 26 130); $\nu_{max.}$ (KBr) 3 000—2 500-br. (CO_2H), 1 675, 1 632, and 1 612 (unsaturated carboxylic acid), and 1 002 cm^{-1} (*trans* CH=CH); δ 1.25—1.85 (4 H, m), 2.0—2.7 (4 H, m), 5.80 (1 H, d, *J* 15 Hz), 5.92 (2 H, s), 5.9—6.6 (2 H, m), 6.4—7.75 (4 H, m), and 10.90br. (1 H, s); *m/z* 274 (M^+, 41), 229 (5), 192 (11), 161 (20), 148 (16), 135 (100), 131 (32), 122 (10), 105 (10), 91 (7), 77 (26), 51 (14), and 39 (9). A sample was converted into the methyl ester with diazomethane in ether–methanol and subjected to g.l.c. (conditions as for previous compound) whereby one main peak (>98%), corresponding to the main component [(2*E*,4*E*)-isomer] of the crude methyl ester (10), was found.

(2E,4E)-N-*Isobutyl*-9-(3,4-*methylenedioxyphenyl*)*nona*-2,4-*dienamide* (3).—A solution of compound (11) (274 mg) in dry ether (10 ml) was treated with oxalyl chloride (2 ml). The mixture was kept under argon for 18 h, with exclusion of moisture. After removal of solvent and excess of oxalyl chloride under reduced pressure, the residue was dissolved in dry ether (25 ml) and the mixture was cooled in an ice-bath. A solution of isobutylamine (1.0 g) in dry ether (25 ml) was gradually added with stirring. The mixture was then washed with dilute hydrochloric acid, water, and aqueous sodium hydrogen carbonate, then dried (Na_2SO_4). Removal of the solvent under reduced pressure afforded a white crystalline residue (0.326 g) which was recrystallised from cyclohexane giving the *amide* (3) (0.253 g), m.p. 114—115 °C (Found: C, 73.0; H, 8.3; N, 4.1. $C_{20}H_{27}NO_3$ requires C, 72.9; H, 8.3; N, 4.2%), identical with an authentic sample of pipercallosine [mixed m.p., u.v., n.m.r., i.r., and mass spectra, and t.l.c. (R_F 0.21)].

N-*Isobutyl*-9-(3,4-*methylenedioxyphenyl*)*nonanamide*.—Compound (3) (82 mg) in ethanol (15 ml) was hydrogenated at room temperature and atm pressure in the presence of pre-reduced platinum oxide (20 mg). Reaction was complete within 75 min, hydrogen (11.5 ml) being taken up. The catalyst was filtered off and the filtrate was evaporated under reduced pressure to afford a crystalline residue (86 mg) which was recrystallised twice from cyclohexane to give the nonanamide (42 mg), m.p. 65—67 °C (Found: C, 72.1; H, 9.5; N, 4.1. $C_{20}H_{31}NO_3$ requires C, 72.0; H, 9.4; N, 4.2%); $\nu_{max.}$ (KBr) 3 360, 1 645, and 1 551 (secondary amide), 2 782, 1 259, 1 043, and 922 cm^{-1} (OCH_2O); δ 0.87 (6 H, d, *J* 6 Hz), 1.0—1.95 (12 H, m), 2.0—2.65 (4 H, m), 2.85—3.25 (2 H, m), 3.87br (1 H, s), 5.85 (2 H, s), and 6.4—6.8 (3 H, m). The mass spectrum was identical with that of hydrogenated pipercallosine (*vide supra*).

Ethyl (2E)-7-(3,4-*Methylenedioxyphenyl*)*hept*-2-*enoate* (12).—Ethyl (diethoxyphosphoryl)acetate (2.47 g, 0.01 mol) was gradually added to an ice-cooled suspension of sodium hydride (0.33 g of an 80% suspension in oil pre-washed with hexane, 0.011 mol) in dry THF (10 ml) under nitrogen. When evolution of gas had ceased, a solution of freshly distilled aldehyde (9) (2.06 g, 0.010 mol) in dry THF (5 ml) was added. After being stirred at room temperature for 2 h, the mixture was poured into water (50 ml) and extracted with ether (3 × 10 ml). The extracts were dried ($MgSO_4$) and evaporated to dryness to afford the crude *ester* (12) as an oil (2.72 g); δ 1.25 (3 H, t, *J* 7 Hz), 1.2—1.9 (4 H, m), 2.0—2.8 (4 H, m), 4.17 (2 H, q, *J* 7 Hz), 5.77 (1 H, d, *J* 15 Hz), 5.87 (2 H, s), and 6.4—7.2 (4 H, m); $\nu_{max.}$ (CCl_4) 1 720 and 1 660 cm^{-1}; $\lambda_{max.}$ (95% EtOH) 209.0 (ε 10 490) and 287.0 nm (3 045). G.l.c. (3% OV 17 on a Gas Chrom Q column at 240 °C) showed a large and a small peak with relative retention times of 1 : 0.81 in the ratio 96 : 4. G.l.c.–mass spectrometry of these two peaks showed them to arise from isomers having very similar mass spectra. The main peak showed *m/z* 276 (M^+, 57), 203 (12), 202 (18), 188 (33), 174 (7), 161 (14), 148 (11), 135 (100), 131 (12), and 122 (6). A specimen for analysis was obtained by distillation, b.p. 145—150 °C at 0.15 mmHg; n_D^{21} 1.5222. G.l.c. indicated this to be a mixture of the (2*E*)- (97%) and the (2*Z*)-isomer (3%) (Found: C, 69.4; H, 7.4. $C_{16}H_{20}O_4$ requires C, 69.5; H, 7.3%).

(2E)-7-(3,4-*Methylenedioxyphenyl*)*hept*-2-*enoic Acid* (13).—Compound (12) (0.90 g) was heated under reflux under nitrogen for 3 h with a 10% solution of potassium hydroxide in 90% aqueous methanol (10 ml). After removal of most of the methanol under reduced pressure, the residue was dissolved in water (20 ml) and the solution was washed with ether. The aqueous phase was acidified with concentrated hydrochloric acid and extracted with ether (2 × 20 ml). The extracts were dried ($MgSO_4$) and evaporated to dryness to afford an oily residue (0.85 g) which solidified. The residue was recrystallised from aqueous ethanol to afford the impure *acid* (13) (0.36 g), m.p. 111—113 °C. A sample was methylated with diazomethane in ether–methanol and the product was investigated by g.l.c. (3% OV 1 on a Gas Chrom Q column at 240 °C). A main peak and two smaller

peaks, with relative retention times 1 : 0.78 : 1.14, were observed in the proportions 91 : 3 : 6. G.l.c.–mass spectrometry of the first two peaks showed that both gave a molecular-ion peak at *m/z* 262, corresponding to that of the (2*E*)- and (2*Z*)-isomer of the methyl ester, while the third peak gave a molecular-ion peak at *m/z* 294, corresponding to that of the methyl ester of a methanol adduct of the unsaturated acid. Further recrystallisation of the acid (13) from aqueous ethanol gave a sample, m.p. 115—116 °C (Found: C, 67.6; H, 6.5. $C_{14}H_{16}O_4$ requires C, 67.7; H, 6.5%); $\lambda_{max.}$ (95% EtOH) 287.5 nm (ε 3 870); $\nu_{max.}$ (KBr) 3 200, 2 400, 1 690, 1 640, and 995 cm^{-1}; δ 1.25—1.95 (4 H, m), 2.0—2.9 (4 H, m), 5.81 (1 H, d, *J* 15 Hz), 5.88 (2 H, s), 6.5—7.4 (4 H, m), and 10.85br. (1 H, s), g.l.c. of the methyl ester of which showed the proportions of the three components to be 99 : 0.5 : 0.5.

(2E)-N-*Isobutyl*-7-(3,4-*methylenedioxyphenyl*)*hept*-2-*enamide* (14).—Oxalyl chloride (3 ml) was added to a solution of compound (13) (0.37 g) in benzene (20 ml) and the solution was heated at 60 °C for 30 min under nitrogen. After removal of excess of oxalyl chloride and solvent under reduced pressure, the residue was dissolved in benzene (10 ml), the solution was cooled in an ice-bath, and isobutylamine (1.5 g) was added with stirring. After 2 h, the solution was washed with 2M hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and water, and then dried ($MgSO_4$). Removal of the solvent under reduced pressure afforded a solid residue (0.47 g) which was triturated with hot cyclohexane. On cooling the cyclohexane solution, a crystalline product (0.19 g) was obtained which was recrystallised from the same solvent to give the *amide* (14) (0.16 g), m.p. 80—82 °C (Found: C 71.2; H, 8.2; N, 4.5. $C_{18}H_{25}NO_3$ requires C 71.3; H 8.3; N, 4.6%), identical (mixed m.p., u.v., n.m.r., i.r., and mass spectra) with an authentic sample of natural pipercallosidine.

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