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STRUCTURAL AND SYNTHETIC STUDIES ON THE RETROFRACTAMIDES—AMIDE CONSTITUENTS OF *PIPER RETROFRACTUM*

AVIJIT BANERJI,* DEBABRATA BANDYOPADHYAY,* MANJUSHA SARKAR,* ARUP K. SIDDHANTA,* SUDHIR C. PAL,* SOMNATH GHOSH,*§ KOSHY ABRAHAM† and JAMES N. SHOOLERY‡

*Pure Chemistry Department, Science College, University of Calcutta, Calcutta 700009, India; †Regional Research Centre, Government Central Pharmacy, Jayanagar, Bangalore 560011, India; ‡Varian Associates, Palo Alto, CA 94303, U.S.A.

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Key Word Index—*Piper retrofractum*; Piperaceae; alkamides; retrofractamides A, C and D; pipericide; sesamin; 3,4,5-trimethoxydihydrocinnamic acid.

Abstract—Two new unsaturated amides, retrofractamides A and C, were isolated from the total above-ground parts of *Piper retrofractum*. Retrofractamide A was shown to be *N*-isobutyl-9(3′,4′-methylenedioxyphenyl)2*E*,4*E*,8*E*-nonatrienamide from spectroscopic and chemical investigations. The structure **6** for retrofractamide C was suggested from spectroscopic and chemical studies and was confirmed by a total stereoselective synthesis. The presence of sesamin and 3,4,5-trimethoxydihydrocinnamic acid as well as two higher homologues of retrofractamide A, viz. pipericide (retrofractamide B) and retrofractamide D was demonstrated. The synthesis of pipericide was also achieved.

INTRODUCTION

Our previous investigations [1–6] on various Indian *Piper* species have led to the isolation and structural elucidation of a number of new compounds from *Piper sylvaticum* Roxb. and *P. aurantiacum* Wall. In continuation of these studies, we took up the investigation of another *Piper* species, viz. *P. retrofractum* Vahl. (syn. *P. chaba* Hunter and *P. officinarum* PC) [7]. Previous reports [7–12] on this species by other groups of investigators have resulted in the isolation of piperine, piperlonguminine, sylvatine, guineensine, piperlongumine, filfiline, sitosterol, fructose, glucose, methyl piperate and mucilage.

RESULTS AND DISCUSSION

The total above-ground parts of *P. retrofractum* were extracted with petrol in a Soxhlet apparatus. The petrol extract on chromatography over silica gel yielded a number of constituents, six of which have been characterized. The first compound, $C_{20}H_{18}O_6$, mp 120° (benzene–petrol, 1:1, eluate) was found to be identical in all respects to sesamin [13]. The second compound, $C_{12}H_{16}O_5$ ($[M]^+$ *m/z* 240), mp 99° (benzene–ethyl acetate, 1:1, eluate) was identified as 3,4,5-trimethoxydihydrocinnamic acid from its spectral properties and by comparison with an authentic sample [14]. This compound has not been reported earlier from any *Piper* species.

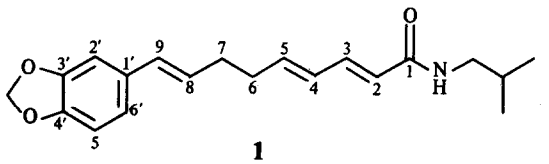
A crude mixture of amides was obtained in the benzene eluates. On careful rechromatography and repeated recrystallization, this afforded the new compound retrofractamide A, whose structure and stereochemistry have been determined as **1**.

Retrofractamide A, $C_{20}H_{25}NO_3$ ($[M]^+$ *m/z* 327), mp 129°, $[\alpha]_D^{25}$ 0° (chloroform, ethanol) showed the UV characteristics (λ_{max}^{EtOH} 262, 211 nm; log ε 4.52, 4.44; λ_{min}^{EtOH} 230 nm; log ε 4.28) of a sorbamide chromophore [15]. Its IR spectrum (KBr) exhibited a band for an –NH– function (3300 cm^{-1}) which was incorporated in a dienamide grouping (1657, 1625 and 998 cm^{-1}). Further bands appeared for a methylenedioxy group (1043, 992 cm^{-1}) and for a 1,2,4-trisubstituted benzene (1615, 870, 815 cm^{-1}). The 200 MHz ^1H NMR spectrum confirmed the presence of a methylenedioxy group (2H, *s*, δ5.90) and an amide proton (1H, *br s*, δ5.3–5.5) and also showed signals for nine aromatic and/or olefinic, and 13 aliphatic protons.

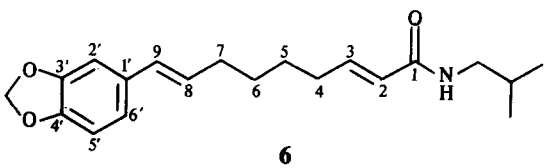
Catalytic hydrogenation of the amide over Adam's catalyst afforded a hexahydro derivative, mp 65°, $C_{20}H_{31}NO_3$ ($[M]^+$ *m/z* 333) whose UV spectrum (λ_{max}^{EtOH} 287, 234 and 216 nm; log ε 3.53, 3.56 and 3.48) was characteristic of a methylenedioxyphenyl moiety [16]. The 80 MHz ^1H NMR spectrum showed the presence of only three protons as a broad signal at δ6.5–6.8. The position of this signal, as well as the appearance of IR bands at 1046, 925 (methylenedioxy) and 868, 813 cm^{-1} (1,2,4-trisubstituted aromatic), indicated that a 3,4-methylenedioxyphenyl moiety was present. The appearance of characteristic mass spectral fragments at *m/z* 148, 136, 135, 107 and 105 corroborated this view [6].

Hence retrofractamide A could be formulated as a trienamide having a 3,4-methylenedioxyphenyl grouping. Detailed 200 MHz ^1H NMR and 20 MHz ^{13}C NMR studies were undertaken for complete structural elaboration of retrofractamide A as *N*-isobutyl-9(3′,4′-methylenedioxyphenyl) 2*E*,4*E*,8*E*-nonatrienamide (**1**). The ^1H NMR spectrum indicated signals characteristic of an *N*-isobutyl moiety, which indicated that the compound was an isobutylamide. In addition, it showed the signals for two allylic methylenes at δ2.00–2.25. Of the six olefinic protons, H-2 and H-3 could be assigned on the basis of the

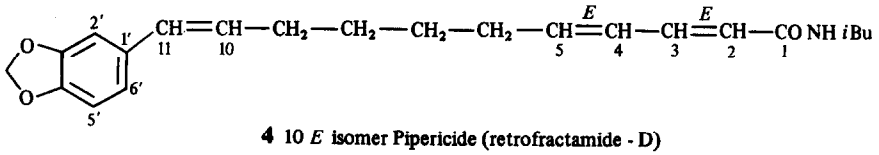
§Present address: Jadavpur University, Calcutta 700032, India.



1
Retrofractamide - A



6
Retrofractamide - C



4 10 *E* isomer Pipericide (retrofractamide - D)

12 10 *Z* isomer

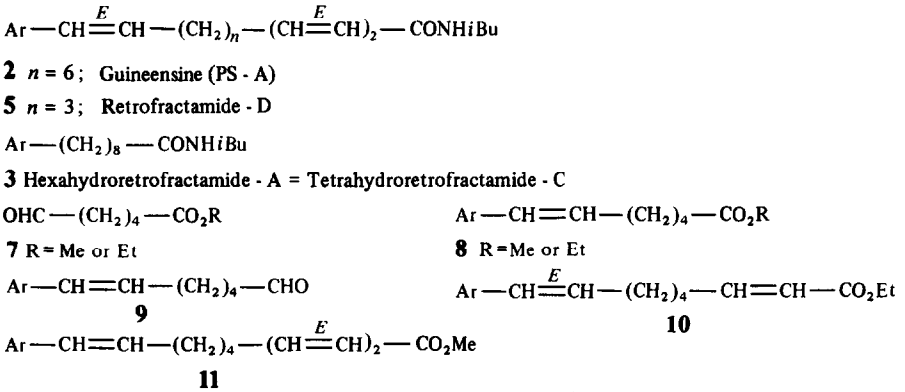
following considerations: H-2 being α to the carbonyl was somewhat upfield and H-3 being β to the carbonyl was very far downfield of the other olefinic protons. In order to simplify the splitting patterns of the olefinic protons and to gain information about the coupling between them, the allylic methylene protons were irradiated. Two of the protons, H-3 and H-4, showed a double-doublet pattern, indicating coupling to two olefinic protons whereas the other four signals had doublet multiplicity. The multiplicity pattern indicated that the third double bond was not conjugated with the dienamide moiety. That it was in the 8,9- rather than the 7,8-position was confirmed by: (i) a subtraction spectrum using an equimolar solution of pipataline [3], a 3,4-methylenedioxystyrene derivative, as blank which gave the unmodified sorbamide UV absorption spectrum (UV: λ_{max} 255, 264 and 273 nm; log ε 4.26, 4.27 and 4.27) [15]; and (ii) the ^{13}C NMR chemical shifts of the aromatic moiety which were similar to those of guineensine (**2**) [6], which also has a 3,4-methylenedioxystyryl moiety. One of the coupling constants of all the olefinic protons was 15–16 Hz. Hence all three double bonds had the *E*-configuration.

The 20 MHz ^{13}C NMR spectrum (chemical shifts in δ, ppm) of retrofractamide A, recorded in $CDCl_3$ solution, also supported structure **1**. The low-field region of the spectrum exhibited an amide carbonyl (166.3, *s* in SFORD spectrum), a methylenedioxy group (100.7, *t*) and 12 olefinic and/or aromatic carbons. Eight of these could be unequivocally assigned by comparison of the chemical shifts with those of guineensine (**2**) [6]: C-8 and C-9 (128.7, *d* and 130.0, *d*), C-1′ (131.9, *s*), C-2′ (105.2, *d*), C-3′ (147.7, *s*), C-4′ (146.4, *s*), C-5′ (108.0, *d*), C-6′ (120.1, *d*). The remaining four olefinic methines at 122.3, 127.4, 140.5 and 141.2 were assigned to the olefinic carbons (C-2 to C-5) of the dienamide grouping. The allylic methylenes resonated at 35.9 (*t*) and 32.6 (*t*) while the isobutyl signal appeared at 28.4 (>CH, *d*), 46.8 (>N–CH_2–, *t*) and 19.9 (two Me groups, *q*).

The mass spectral fragmentation patterns of retrofractamide A (**1**) and hexahydroretrofractamide A (**3**) were in agreement with the proposed structures. These were very similar to those of guineensine (PS-A) [6], a higher homologue of retrofractamide A.

The mass spectrum of the crude mixture of amides showed the presence of several components, having $[M]^+$ peaks at *m/z* 357, 355 (pipericide, initially designated retrofractamide B), 353, 345, 343, 341 (retrofractamide D) and 329 (retrofractamide C) in addition to retrofractamide A (327), which was the major component.

Careful fractionation of this crude mixture yielded pure retrofractamides A and C in addition to a less complex mixture of retrofractamides A, B and D in a ratio of *ca* 15:5:2, further purification of which could not be carried out. The spectral properties (UV, IR, ^1H NMR) of this mixture were virtually identical to those of retrofractamide A, except for the presence of an additional broad signal at δ1.3–1.5 corresponding to non-allylic aliphatic methylenes in the ^1H NMR spectrum. Mass spectral analysis of this mixture revealed $[M]^+$ peaks at *m/z* 327, 341 and 355. Hydrogenation of this mixture gave a



Ar = 3,4 - methylenedioxyphenyl

mixture of hexahydro derivatives ($[M]^+$ *m/z* 333, 347, 361). Except for the additional $[M]^+$ peaks at *m/z* 361 and 347 and an enhanced integration for the methylene protons in the ^1H NMR spectrum, the spectral properties of the mixture of the reduced compounds were very similar to those of hexahydroretrofractamide A. It thus seemed that retrofractamide B (**4**) and retrofractamide D (**5**) are higher homologues of retrofractamide A, containing two and one additional methylene groups, respectively. The former (**4**) thus appears to be identical to pipericide, an alkamide reported from *P. nigrum* [17].

Retrofractamide C, $C_{20}H_{27}NO_3$, mp 120°, $[\alpha]_D^{25}$ 0° (chloroform, ethanol) exhibited the UV characteristics (λ_{max}^{EtOH} 217, 233 and 289.5 nm; log ε 4.00, 3.90 and 3.87) of an unsaturated amide. Its IR spectrum (KBr) showed characteristic bands for an –NH– incorporated in an α,β-unsaturated amide moiety (3250, 1650, 1600 cm^{-1}), a methylenedioxy grouping (1250, 1030, 930 cm^{-1}) and a *trans*-double bond (960 cm^{-1}). The 80 MHz ^1H NMR spectrum confirmed the presence of the methylenedioxy (δ 5.87, 2H, *s*) and the isobutylamide (*i*-Bu NH) (δ 5.60, 1H, *br s*, disappearing on deuteration; δ 0.90, 6H, *d*, *J* = 6.3 Hz; δ 3.12, 2H, *d*, *J* = 6.3 Hz; δ 1.70, 1H, partially obscured *m*) groups. Moreover, it showed signals for seven aromatic and/or olefinic protons (δ 5.60–6.40, 3H, *m* and δ 6.60–7.05, 4H, *m*) and four aliphatic methylene groups, of which two were allylic (δ 2.15–2.40, 4H, *br m*) and two non-allylic (δ 1.35–1.60, 4H, *br m*).

Catalytic hydrogenation of the amide over Adam's catalyst afforded a tetrahydro derivative, $C_{20}H_{31}NO_3$ ($[M]^+$ *m/z* 333), mp 65°. This compound was found to be identical to hexahydroretrofractamide A (**3**). Hence retrofractamide C had the same carbon skeleton as retrofractamide A and differed from the latter in having two instead of three olefinic bonds. One of these is in conjugation with the carbonyl group, as indicated by its IR spectrum. The presence of ^1H NMR signals for only two allylic/benzylic methylenes in retrofractamide C suggests that the second double bond is either at the 4,5 or 8,9-position. The position and stereochemistry of the double bonds were determined from a detailed study of the 20 MHz ^{13}C NMR spectrum and comparison of the chemical shifts with the compounds pipataline [3], dihydropipataline and *N*-isobutyl-2*E*-decenamide [5]. It was observed that the chemical shifts of the aromatic carbons of the unknown amide were similar to the corresponding values of pipataline rather than to those of dihydropipataline. This indicated that a 3,4-methylenedioxystyryl moiety was present in retrofractamide C. Hence the second double bond was present at the 8,9-position and its configuration was *E*. Thus retrofractamide C should have structure **6**. The close correspondence in chemical shifts of the four olefinic carbons of **6** to those of pipataline and *N*-isobutyl-2*E*-decenamide [5] further suggested that both double bonds in **6** had *E*-stercochemistry. All the ^{13}C NMR assignments are listed in Table 1.

The structure and stereochemistry (**6**) thus assigned to retrofractamide C were confirmed by its total stereoselective synthesis as described below. Ethyl or methyl 6-oxo-hexanoate (**7**, R = Et or Me) was used to incorporate the central 6-carbon unit (C-3 to C-8). It was prepared from ε-caprolactone, itself obtained by a Baeyer–Villiger ring expansion of cyclohexanone, by acid-catalysed ethanolysis followed by oxidation with pyridinium chlorochromate. In an alternative method, ε-caprolactone was treated with 48 % HBr–H_2SO_4, and the product esterified with diazomethane. The resulting methyl 6-bromohexanoate was then converted into the tosylate, which was oxidized to **7**, (R = Me) by DMSO–$NaHCO_3$ [18]. The 8,9-double bond was stereoselectively generated by a Wittig reaction of triphenyl piperonyl phosphonium bromide and **7** (R = Et or Me). This reaction occurred with greater stereoselectivity in DMSO (*E*:*Z* ratio 9:1)

Table 1. ^{13}C NMR chemical shift assignments of retrofractamide C and related compounds

C	1	Pipataline	Dihydro-pipataline	*N*-Isobutyl 2*E*-decenamide
1	166.3 (*s*)	—	—	166.2 (*s*)
2	123.7 (*d*)	—	—	122.2 (*d*)
3	143.9 (*d*)	—	—	142.1 (*d*)
4	32.4* (*t*)	—	—	
5	27.6† (*t*)	—	—	
6	28.7† (*t*)	—	—	
7	31.6* (*t*)	—	—	
8	129.4‡ (*d*)	C-10‡ 129.1	—	
9	128.7‡ (*d*)	C-11‡ 129.3		
1′	131.9 (*s*)	132.4 (*s*)	136.7 (*s*)	
2′	105.2 (*d*)	105.3 (*d*)	107.8 (*d*)	
3′	147.7 (*s*)	147.8 (*s*)	147.3 (*s*)	
4′	146.4 (*s*)	146.4 (*s*)	145.3 (*s*)	
5′	108.0 (*d*)	108.0 (*d*)	108.7 (*d*)	
6′	120.0 (*d*)	120.0 (*d*)	120.8 (*d*)	
1″	46.8 (*t*)	—	—	
2″	28.4 (*d*)	—	—	
3″, 4″	19.9 (*q*)	—	—	
–O–CH_2O–	100.7 (*t*)	—	—	

*, †, ‡These pairs of values may be interchanged.

than in DMF (*E*:*Z* ratio 3:2) as solvent. The unsaturated ester (**8**, R = Et), obtained from the reaction in DMSO, was converted to the aldehyde (**9**). The carbon skeleton of the molecule was finally constructed by stereoselective generation of the 2,3-double bond by the Wittig–Horner–Emmons reaction [19–21] of **9** with enethyl phosphonoacetate. The resulting dienoate ester (**10**) was then converted to the corresponding isobutylamide (**6**) by the reaction sequence shown. The synthetic compound was identical in all respects to the natural product. Hence the structure and stereochemistry of retrofractamide C were established unequivocally as **6**.

Pipericide has also been synthesized in the course of the present work. The unsaturated aldehyde (**9**), a key intermediate in this synthesis, was obtained from the ~ 3:2 = *E*:*Z* mixture of **8** (R = Et) (obtained from the Wittig reaction in DMF mentioned earlier) by a sequence of reactions similar to those described earlier for retrofractamide C. A Wittig–Horner–Emmons condensation of **9** with methyl-4-diethyl-phosphonocrotonate stereoselectively generated the 2*E*,4*E*-dienoate system to give **11**. This was then converted to the desired isobutylamide via the acid chloride. The product, a white crystalline solid, mp 142–43°, was shown to be a mixture of the 10*E* (**4**) and 10*Z* (**12**) isomers, in the ratio 11:9, by NMR analysis. A small amount of pure 10*E*-isomer (**4**), mp 114°, was separated from the mixture by repeated preparative TLC over silver-nitrate impregnated silica gel. Thus this constitutes a synthesis of pipericide (**4**).

EXPERIMENTAL

Mps were recorded on a Kofler block and are uncorr. ^{1}H NMR spectra were recorded at 80 or at 200 MHz while ^{13}C NMR spectra were taken at 20 MHz. Analytical samples were routinely dried at 30° *in vacuo*. Dry Na_2SO_4 was used for drying extracts. CC and TLC were carried out using silica gel (60–120 mesh) and silica gel G, respectively.

Plant material. P. retrofractum Vahl. was collected in Kerala. A voucher specimen (PR-WP) has been preserved in our laboratory.

Isolation. Crushed and dried aerial parts of *P. retrofractum* (2 kg) were extracted with petrol (bp 60–80°) (8 l.) for 80 hr in a Soxhlet apparatus. The petrol extract was concd and chromatographed over silica gel. The petrol–C_6H_6 (2:1) eluate furnished sesamin, mp 120°, which was identified by comparison (mp, mmp, co-TLC, superimposable IR and ^{1}H NMR spectra) with an authentic sample [13]. A mixture of closely related amide derivatives was obtained in the C_6H_6 eluate. This mixture was subjected to repeated chromatography and prep. TLC to obtain retrofractamide A, mp 129°, $[\alpha]_D^{25}$ 0° ($CHCl_3$, EtOH) and retrofractamide C, mp 120°, $[\alpha]_D^{25}$ 0° ($CHCl_3$, EtOH). A less complex mixture of the amides mp 115–125°, was also obtained. MS analysis (15 eV) showed the presence of three $[M]^+$ peaks at *m/z* 327, 341 and 355, the ratio of peak intensities being 15:2:5. The C_6H_6–EtOAc (4:1) eluate furnished trimethoxyphenylacetic acid, mp 99°. Found: C, 74.8, H, 8.2 Calc. for $C_{12}H_{16}O_2$: C, 75.0; H, 8.4%. IR ν_{max}^{KBr} cm^{-1}: 3200 (–OH), 1688 (–CO_2H), 652, 744, 760, 850, 1450, 1505 (1,2,3,5-tetrasubstituted benzene). 80 MHz ^{1}H NMR ($CDCl_3$): δ6.5–6.7 (*br s*, exchangeable with D_2O, –CO_2H), 6.39 (*s*, Ar-H), 3.82 (6H, *s*, –OMe), 3.80 (3H, *s*, –OMe), 2.65–2.89 (*m*, –CH_2–CH_2–). MS (70 eV) *m/z*: 240 $[M]^+$, 194 $[M - CO_2H]^+$, 179 $([M - CO_2H - Me]^+)$, 181.

Retrofractamide A. Found: C, 73.1; H, 7.8; N, 4.1. $C_{20}H_{25}NO_3$ requires: C, 73.4; H, 7.7; N, 4.3%. 200 MHz ^{1}H NMR ($CDCl_3$): δ7.17 (*dd* with *f.s.*, *J* = 15, 10 Hz, H-3), 6.86 (*br s* with *f.s.*, H-6'), 6.71 (*br s* with *f.s.*, H-2' and H-5'), 6.28 (*d**, *J* = 16 Hz, H-9), 6.14 (*dd**, *J* = 15, 10 Hz, H-4), 6.07 (*d**, *J* = 15 Hz, H-5), 5.99 (*d**, *J* = 16 Hz, H-8), 5.90 (*s*, –O–CH_2–O–), 5.74 (*d*, *J* = 15 Hz, H-2), 5.30–5.50 (*br s*, –NH–), 3.15 (*t*, *J* = 7 Hz, –NH–C$\underline{H}_2$–), 2.00–2.25 (*m*, allylic –CH_2–) 1.75 [9 line, *J* = 7 Hz, –C$\underline{H}Me_2$], 0.90 [*d*, *J* = 7 Hz, –$CHMe_2$]. MS (70 eV) *m/z*: $[M]^+$ 327, 312 $[M - Me]^+$, 299 $[M - CO]^+$, 256 $[M - NH\text{-}i\text{-}Bu + H^-]^+$, 255 $[M - NH\text{-}i\text{-}Bu]^+$, 228, 227, 161, 152, 148, 136, 135, 133, 131, 107, 105, 100, 72.

Retrofractamide C. Found: C, 72.6; H, 8.5; N, 4.1. Calc. for $C_{20}H_{27}NO_3$: C, 72.9; H, 8.3; N, 4.3%. UV λ_{max}^{EtOH} nm (log ε): 217 (4.00), 233 (3.90), 289.5 (3.87), 320.5 (3.90). IR ν_{max}^{KBr} cm^{-1}: 3250 (–NH–), 1650, 1600 (α,β-unsaturated amide), 960 (–CH=$\underline{E}$CH–), 1250, 1030, 930 (–OCH_2O–), 800, 680 (trisubstituted aromatic ring).

Hydrogenation of retrofractamide A. Retrofractamide A (20 mg) in EtOH soln (10 ml) was hydrogenated under atmos. pres. over Adam's catalyst. After the uptake of H_2 was complete, the reaction mixture was filtered and evapd to dryness. The residue was crystallized from MeOH to yield hexahydroretrofractamide A (**3**), mp 65°. Found: C, 73.1; H, 9.8; N, 4.1. $C_{20}H_{33}NO_3$ requires: C, 73.4; H, 10.1; N, 4.3%. 80 MHz ^{1}H NMR ($CDCl_3$): δ6.5–6.8 (*s*, Ar-H), 5.87 (*s*, –OCH_2O–), 5.60–5.75 (*br s*, NH), 3.05 (*t*, *J* = 6.3 Hz, NHC$\underline{H}_2$), 2.25–2.55 (*m*, C-9 H_2), 1.00–2.35 (*m*, C-2 to C-8 methylenes and C$\underline{H}Me_2$, 0.88 (*d*, *J* = 6.3 Hz, –CH(C$\underline{H}_3$)$_2$). MS (70 eV) *m/z*: $[M]^+$ 333, 290 $[M - i\text{-}Pr]^+$, 277 $[M - i\text{-}Bu + H^-]^+$, 261 $[M - NH\text{-}i\text{-}Bu]^+$, 232, 218, 198, 190, 162, 148, 136, 135, 128, 115, 107, 105, 100, 72.

Hydrogenation of retrofractamide C. Retrofractamide C was hydrogenated under conditions similar to those described above. Tetrahydroretrofractamide C, mp 65° (MeOH), was found to be identical to hexahydroretrofractamide A by direct comparison (IR, ^{1}H NMR, MS, mixed mp and co-TLC).

Ethyl 6-hydroxyhexanoate. A soln of cyclohexanone (7.8 g, 80 mmol) in dry CH_2Cl_2 (20 ml) was added to a cold soln of *m*-chloroperbenzoic acid (17.2 g, 100 mmol) in dry CH_2Cl_2 (100 ml) at 0°. The mixture was kept at 0° for 10 days. The precipitated *m*-chlorobenzoic acid was filtered off. The filtrate was concd, and the residual liquid on distillation under red. pres. afforded ε-caprolactone, bp 98–99°/12 mm (lit. [22] 96–97.5°/10 mm). This method was superior to that reported in ref. [22].

ε-Caprolactone was dissolved in EtOH (50 ml) containing 4 ml conc. H_2SO_4 and the reaction mixture refluxed for 6 hr. The liquid was diluted with H_2O (100 ml) and extracted with Et_2O (3 × 50 ml). The crude product obtained on removal of solvent was purified by distillation under red. pres., bp 134–135°/15 mm (5.0 g, 41%); Found: C, 59.7; H, 10.3. Calc. for $C_8H_{16}O_3$: C, 59.9; H, 10.1%). IR ν_{max} cm^{-1}: 3425 (OH), 1730 (C=O). 80 MHz ^{1}H NMR ($CDCl_3$): δ1.18 (*t*, *J* = 7 Hz, –Me), *ca* 1.45 (*m*, C-3, C-5 methylenes), 2.23 (*t*, *J* = 6.7 Hz, C-2 H_2), 2.39 (*s*, –OH), 3.53 (*t*, *J* = 6.2 Hz, C-6 H_2), 4.03 (*q*, *J* = 7 Hz, –OC$\underline{H}_2$Me).

Ethyl 6-oxohexanoate (**7**, R = Et). Ethyl 6-hydroxyhexanoate (4 g, 25 mmol) in dry CH_2Cl_2 (10 ml) was added in one portion to a suspension of pyridinium chlorochromate (8.01 g, 37.5 mmol) in dry CH_2Cl_2 (50 ml) and the mixture stirred for 1.5 hr at 25° [23]. Dry Et_2O (100 ml) was added and the supernatant liquid decanted off. The residue was washed with Et_2O (4 × 50 ml). The combined organic extracts were filtered through Celite and the solvent was removed. The residue was distilled to yield **7** (R = Et), bp 100°/10 mm (3.5 g, 89%). IR ν_{max} cm^{-1}: 1730 (C=O). 80 MHz ^{1}H NMR ($CDCl_3$): δ9.67 (*t*, *J* = 1.6 Hz, CHO), 4.05 (*q*,

*Note: Splitting patterns for H-4, H-5, H-8, H-9 are those obtained on decoupling of allylic protons. Before decoupling a complex miltiplet comprising H-4, H-5, H-8 and H-9 was obtained.

J = 7.2 Hz, –O–CH_2–), *ca* 2.25 (*m*, C-3, C-4 methylenes), *ca* 1.60 (*m*, C-2, C-5 methylenes), 1.19 (*t*, J = 7.2 Hz, –Me).

Methyl 6-oxo-hexanoate (**7**; R = Me). Methyl 6-bromo-hexanoate, bp 115–120°/8–9 mm, was obtained from ε-caprolactone by a method similar to that described for the corresponding ethyl ester [24]. This bromo-ester (5 g) was added to a soln of AgOTs (8 g) in MeCN (80 ml) at 0–5° in the dark. After being kept overnight at room temp., the mixture was added to ice-H_2O and then extracted with Et_2O. Removal of solvent from the dried extract gave an oil that was oxidized [18] by a freshly prepared mixture of $NaHCO_3$ (15 g) and Me_2SO_4 (100 ml) preheated at 150° under N_2 in 3 min. The mixture was rapidly cooled to room temp. and then diluted with H_2O. Extraction with Et_2O followed by chromatography of the concentrate furnished the product **7** (R = Me), 1.8 g, in the C_6H_6 eluate.

Ethyl-7(3',4'-methylenedioxyphenyl)6-heptenoate (**8**; R = Et). Piperonyl bromide (8.4 g, 36 mmol) and triphenyl phosphine (9.4 g, 36 mmol) were refluxed in dry C_6H_6 (50 ml) for 1 hr, when triphenyl piperonyl phosphonium bromide precipitated out. This was dried *in vacuo* and stored in a sealed tube. Triphenyl piperonyl phosphonium bromide (9.5 g, 24 mmol) was added with stirring under N_2 to a suspension of NaH (50%) (1.2 g, 24 mmol) and dry DMSO (30 ml) at 0°. After 10 min, ethyl 6-oxo-hexanoate (**7**, R = Et) (3.2 g, 20 mmol) in 5 ml DMSO was added dropwise to the orange-red soln with stirring at 25° over 45 min, whereby the colour began to disappear gradually. Stirring was continued for 8 hr. The mixture was left overnight at ~ 25°, then diluted with H_2O (30 ml) and extracted with Et_2O (4 × 50 ml). The Et_2O layer was washed with H_2O, dried and distilled. The residue on chromatography yielded **8** (R = Et) as an oily liquid (2.5 g, 35% yield). Found: C, 69.8; H, 7.3; $[M]^+$ m/z 276. $C_{16}H_{20}O_4$ requires: C, 69.6; H, 7.2%; $[M]^+$ m/z 276. IR ν_{max} cm^{-1}: 1730 (C=O); 930, 920 (*E*-styryl double bond). 80 MHz ^1H NMR ($CDCl_3$): δ1.25 (*t*, J = 6 Hz, –Me), 1.40–1.90 (*m*, C-3, C-4 methylenes), 2.00–2.36 (*m*, C-2, C-5 methylenes), 4.10 (*q*, J = 6 Hz, –C<u>H</u>$_2$–Me), 5.85 (*s*, –OCH_2O–), 6.00 (*dt*, J = 16, 7.5 Hz, C-6H), 6.28 (*d*, J = 16 Hz, C-7H), 6.55–6.80 (*m*, aromatic H's); *Z*-isomer: 5.4–5.8 (*m*, C-6 H), 6.30 (*d*, J = 10 Hz; C-7 H). Integration ratio of C-6 H (*Z*-isomer) to C-7H (*E* + *Z*-isomers) is 1:10, corresponding to an *E*:*Z* ratio of 9:1.

The reaction was also carried out using DMF as reaction solvent, under similar conditions. ^1H NMR analysis of the ester (**8**) formed showed an integration ratio of C-6H (*Z*-isomer) to C-7H (*E* + *Z*-isomers) of 5:12 corresponding to an *E*:*Z* ratio of 58:42. The Wittig–Horner–Emmons reaction [19–21] of **7** (R = Me) and diethyl phosphonocrotonate at 110° in diglyme proceeded stereospecifically but the overall yield was extremely poor (8%).

7(3',4'-Methylenedioxyphenyl)-hept-6-en-1-ol. To a well-stirred suspension of $LiAlH_4$ (600 mg) in dry C_6H_6 (30 ml), **8** (R = Et) (1.4 g, 5 mmol) in C_6H_6 (10 ml) was added and the mixture heated at 60° for 8 hr. The complex was decomposed with sodium–potassium tartrate soln and extracted with Et_2O (3 × 100 ml). Removal of solvent gave the desired product as a semi-solid mass (800 mg, 67% yield). IR ν_{max}^{Nujol} cm^{-1}: 3350 (–OH), 1600 (C=C), 935 (*E*-double bond). 80 MHz ^1H NMR ($CDCl_3$): δ3.46 (*t*, J = 6 Hz, –C<u>H</u>$_2$OH).

7(3',4'-Methylenedioxyphenyl)-6-heptenal (**9**). The alcohol (702 mg, 3 mmol) obtained in the previous step was oxidized with pyridinium chlorochromate (968 mg, 4.5 mmol) by the procedure described for **7** (R = Et) [23]. The crude product was chromatographed over silica gel to yield **9** (350 mg, 50% yield) as a semi-solid mass with C_6H_6–EtOAc (9:1) as eluant. IR ν_{max} cm^{-1}: 1720, 1680 (C=O), 1600 (C=C), 925 (*E*-double bond). 80 MHz ^1H NMR ($CDCl_3$): δ9.74 (1H, *t*, –C<u>H</u>O).

Ethyl-9(3',4'-methylenedioxyphenyl)-2E,8E-nonadienoate (**10**). Triethyl phosphonoacetate was prepared by the Michaelis–Arbuzov reaction according to refs. [19–21]. The phosphonoacetate (336 mg, 1.5 mmol) was added to a slurry of NaH (72 mg, 1.5 mmol) in dry diglyme (20 ml) at 20°, and the mixture stirred for 1 hr; **9** (348 mg, 1.5 mmol) was added dropwise at such a rate that the temp. was maintained below 30°. The reaction mixture was stirred at 25–30° for 4 hr and then at 60–70° for 5 hr during which time a gelatinous solid appeared. The reaction mixture was poured into a saturated soln of sodium–potassium tartrate (150 ml) and left for 2 hr. It was then extracted with Et_2O (3 × 100 ml). The Et_2O layer was washed several times with H_2O, dried and evapd to obtain the diolefinic ester (**10**), R_f 0.25 in petrol–C_6H_6 (1:1) (150 mg, 33%). IR ν_{max} cm^{-1}: 1710 (C=O), 1605, 980 (*E*-double bond). **10** was used in the subsequent steps without further purification.

9(3',4'-Methylenedioxyphenyl)-2E,8E-nonadienoic acid. To a soln of **10** (45 mg, 0.15 mol) in MeOH (10 ml) was added 10% NaOH (1 ml) and the mixture refluxed for 1 hr. MeOH was removed and the residue diluted with H_2O and washed with Et_2O. The aq. layer was acidified with 6 M HCl, when a pale yellow solid appeared. This was filtered off and washed (30 mg, 73% yield). IR ν_{max} cm^{-1}: 3465 (–OH), 1710 (C=C), 985 (*E*-double bond), 930 (–OCH_2O–).

N-Isobutyl- 9(3',4'- methylenedioxyphenyl)- 2E,8E- nonadienamide (**6**). To a suspension of the acid (27.4 mg, 0.1 mmol) in dry C_6H_6 (5 ml) was added an excess of oxalyl chloride (25.0 mg, ~ 0.13 mmol) with stirring at 0° for 10 min under dry N_2. The stirring was continued for 30 min at 25° and for 1 hr at 60°. Excess oxalyl chloride and C_6H_6 were removed *in vacuo*. The acid chloride thus formed was dissolved in dry Et_2O (10 ml). Isobutylamine (18.2 mg, 0.25 mmol) in Et_2O (5 ml) was added dropwise with cooling and shaking. After 1 hr, the soln was washed with H_2O, dried and evapd. The residue was purified by CC over silica gel. A white crystalline solid, mp 120°, R_f 0.40 in C_6H_6–EtOAc (4:1), was obtained using C_6H_6 as eluant (5 mg, 7% yield). Found: C, 72.8; H, 8.5; N, 4.1 ($[M]^+$ m/z 329). Calc. for $C_{20}H_{27}NO_3$: C, 72.9; H, 8.3; N, 4.3%). Its spectroscopic properties (UV, IR, ^1H NMR, ^{13}C NMR, MS) were identical to those of the naturally occurring retrofractamide C (**6**).

Methyl 11-(3',4'-methylenedioxyphenyl)-undeca-2,4,10-trienoate (**11**). Methyl 4-diethylphosphonocrotonate was prepared according to the method described earlier by us [1]. This was added to a slurry of NaH (110 mg of a 55–60% suspension in mineral oil) in dry DMF (15 ml) at 5° under N_2, and the resulting mixture stirred for 30 min. The aldehyde (**9**) (*ca* 3:2 = *E*:*Z* mixture) (600 mg) was then added slowly, the mixture was stirred for 2 hr at 5° and then allowed to reach room temp. at which it was kept for another 15 hr. This was then diluted with H_2O and extracted with Et_2O. The Et_2O extract was dried and concd. The residue on chromatography over neutral Al_2O_3 afforded the ester (**11**) as a colourless oil (325 mg) in the petrol–C_6H_6 (1:1) eluate. IR ν_{max} cm^{-1}: 1700 (ester CO); 1605, 998, 925 (*E*-double bond).

Pipericide (**4**) *and* **12**. A soln of the ester (**11**) in EtOH (25 ml) containing KOH (2.0 g) was refluxed for 3 hr. The solvent was removed, H_2O was added and the soln acidified with 3 M HCl. The ppt. was filtered off, washed with H_2O and dried. A soln of the acid in dry C_6H_6 (10 ml) was treated with oxalyl chloride (0.15 g) at 25° for 15 min. This was followed by refluxing for 20 min. The brown oily residue, obtained on removal of solvent and excess of the reagent, was dissolved in dry Et_2O (10 ml). Isobutylamine (0.11 g) in Et_2O (5 ml) was added dropwise with stirring. After 15 min, the mixture was poured into H_2O and extracted with Et_2O. The organic extract was washed successively with 0.5 M H_2SO_4, aq. $NaHCO_3$ and H_2O. Chromatography on silica gel of the concentrate gave a solid (110 mg), mp 142–143°, in the C_6H_6–EtOAc (19:1) eluate. 200 MHz ^1H NMR analysis

showed this to be a 55:45 mixture of the $\Delta^{10,11}$ *E* (**4**) and *Z* (**12**) isomers. A small amount of **4**, mp 114°, could be obtained in the pure state by repeated prep. TLC over $AgNO_3$-impregnated silica gel **4**. UV λ_{max}^{EtOH} nm: 216 (4.84), 260 (4.75); IR ν_{max}^{KBr} cm^{-1}: 3315 (–NH–), 1655 (conjugated C=O) and 998 (*E*-double bond). 200 MHz ^1H NMR ($CDCl_3$) (decoupled by irradiation of allylic methylenes at δ2.00–2.45): δ7.15 (*dd*, J = 15, 9 Hz, H-3), 6.60–6.87 (*m*, aromatic H's), 6.30 (*d*, J = 16 Hz, H-11), 6.12 (*dd*, J = 16, 9 Hz, H-4), 6.03 (*d*, J = 16 Hz, H-10), 6.06 (*d*, J = 16 Hz, H-5), 5.93 (*s*, $-OCH_2O-$), 5.75 (*d*, J = 15 Hz, H-2), *ca* 5.5 (*br*, –NH–), 3.16 (*t*, J = 6 Hz, $-N-C\underline{H}_2-$), *ca* 1.75 (*m*, $-C\underline{H}Me_2$), 1.25–1.60 (*m*, C-7, C-8 methylenes), 0.93 (*d*, J = 6 Hz, $-CH\underline{Me}_2$).

12. 200 MHz ^1H NMR ($CDCl_3$) (decoupled by irradiation of allylic methylenes at δ2.00–2.45; detected in a mixture with **4**): δ7.16 (*dd*, J = 15, 9 Hz, H-3), 6.60–6.87 (*m*, aromatic H's), 6.32 (*d*, J = 11 Hz, H-11), 6.14 (*dd*, J = 16, 9 Hz, H-4), 5.97–6.12, (obscured by overlap with other signals, H-5), 5.95 (*s*, $-OCH_2O-$), 5.75 (*d*, J = 15 Hz, H-2), *ca* 5.5 (*br*, –NH–), 5.55 (*d*, J = 11 Hz, H-10); other signals identical to those of **4**.

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