

AN AMIDE FROM FRUITS OF *PIPER NIGRUM*

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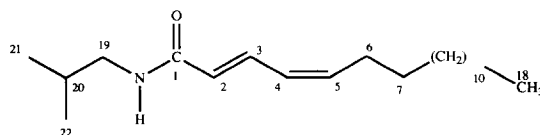
Key Word Index—*Piper nigrum*; Piperaceae; *N*-isobutylamide of octadeca-*trans*-2-*cis*-4-dienoic acid; piperine; 2D NMR.**Abstract**—A new amide, *N*-isobutyl amide of octadeca-*trans*-2-*cis*-4-dienoic acid has been isolated from the dried and crushed fruits of *Piper nigrum*, along with the known alkaloid, piperine. Complete assignments of the protons and carbons of the new amide and piperine have been made based on 2D NMR studies. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

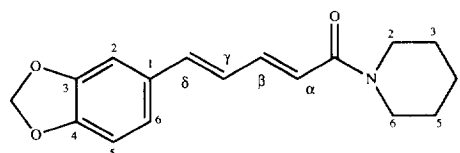
Piper nigrum, commonly known as black pepper, is a climbing perennial shrub. The fruit is dark green at first passing through orange-yellow to dull red when ripe. Black pepper is known to be acrid, pungent and hot. Internally, it is used as a stimulant and carminative, and induces secretion of bile. Externally, it is a rubefacient and stimulant to the skin. It is also prescribed for cholera, dyspepsia, flatulence, diarrhoea, various gastric ailments and for paralytic and arthritic disorders [1–4]. Several alkaloidal and non-alkaloidal constituents have been reported from time to time [5]. This report describes the isolation and structure elucidation of a new amide, pipericine (1), along with piperine (2). Structural determination is based on spectral methods. The ^{13}C NMR shifts of piperine agree well with those reported earlier [6].

RESULTS AND DISCUSSION

The molecular formula of 1, $\text{C}_{22}\text{H}_{41}\text{NO}$, was obtained through a combined application of EI mass spectrometry ($[\text{M}]^+$, m/z 335) and HREI-mass spectrometry (m/z 335.3317). The IR spectrum showed characteristic bands for $-\text{NH}$ (3300 , 3100 cm^{-1}), $\text{C}=\text{O}$ (1654 cm^{-1}), $\text{C}=\text{C}$ (1625 cm^{-1}) and $\text{CH}=\text{CH}$ (998 and 965 cm^{-1}). The UV spectrum had absorption at 256.8 nm . These data indicated the presence of a *trans*-2-*cis*-4-dienamide system [7]. The ^1H NMR spectrum showed three methyls, one as a triplet at δ 0.85 ($J = 7.20\text{ Hz}$, H-18) and two as doublets at δ 0.93 (6H, $J = 6.20\text{ Hz}$, H-21, H-22), a broad



Pipericine (1)



Piperine (2)

singlet at δ 1.25 (16H) due to methylene protons, a one proton nine-line pattern at δ 1.79 for CH (H-20) of an isopropyl terminal, two one proton multiplets at δ 2.13 and 2.32 attributable to the allylic protons (H-6a and H-6b) and a triplet of two protons centred at δ 3.15 (H-19) accountable for $-\text{NH}-\text{CH}_2-\text{CH}$ typical of isobutylamides [8], which changed to a doublet on shaking with D_2O . The ^1H NMR further displayed a one-proton doublet at δ 5.70 ($J = 15.6\text{ Hz}$, H-2), two one-proton double doublets at δ 7.10 ($J = 15.6$, 9.0 Hz , H-3) and 6.08 ($J = 12.1$, 9.0 Hz , H-4), and a one-proton multiplet at δ 5.95 (H-5). These assignments are based on the chemical shifts, coupling constants, proton-proton decoupling experiments and interactions observed in the COSY 45 plot, and agree well with the reported values of *trans*-2-*cis*-4-dienone systems [9]. In the HMQC plot, H-2, H-3, H-4 and H-5 showed their connectivities with C-2 (δ 120.0), C-3 (δ 139.0), C-4 (δ 124.0) and C-5 (δ 114.5), respectively.

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Table 1. ^{13}C and ^1H NMR data of pipericine (1). Quaternary C signals assigned by DEPT. Proton signals assigned on the basis of ^1H - ^1H -decoupling and COSY-45 plot. $^1\text{H}/^{13}\text{C}$ relationship was established on the basis of cross-peaks observed in the HMQC spectrum

Carbon no.	δ_{C}	Correlated protons δ_{H}	Multiplicity	J/Hz
1	166.0	—	—	—
2	120.0	5.70	<i>d</i>	5.7
3	139.0	7.10	<i>dd</i>	15.6, 9.6
4	124.0	6.08	<i>dd</i>	12.1, 9.0
5	114.5	5.95	<i>m</i>	—
6	34.0	2.13	<i>m</i>	—
		2.32	<i>m</i>	—
7	31.5	1.31	<i>m</i>	—
8	29.5	1.25	<i>m</i>	—
9	29.5	1.25	—	—
10	29.5	1.25	—	—
11	29.5	1.25	—	—
12	29.5	1.25	—	—
13	29.5	1.25	—	—
14	29.5	1.25	—	—
15	29.5	1.25	—	—
16	31.5	1.31	<i>m</i>	—
17	23.0	1.30	<i>m</i>	—
18	14.0	0.85	<i>t</i>	7.2
19	47.5	3.15	<i>t</i>	6.1
20	25.1	1.79	<i>m</i>	—
21	22.6	0.93	<i>d</i>	6.2
22	22.6	0.93	<i>d</i>	6.2

The mass fragments (*vide* structure) at m/z 278, 263.2352 ($\text{C}_{18}\text{H}_{31}\text{O}$), 235.2042 ($\text{C}_{16}\text{H}_{27}\text{O}$), 166.1254 ($\text{C}_{10}\text{H}_{16}\text{NO}$), 85.0965 (C_6H_{11}) and 71.0827 (C_5H_{11}) supported the structure of **1** as the *N*-isobutylamide of octadeca-*trans*-2-*cis*-4-dienoic acid. The structure was substantiated by the ^{13}C NMR (broad band and DEPT) (Table 1) which showed three methyls, four olefinic CH, one sp^3 CH, one carbonyl, two downfield methylenes (C-19 and C-6) and 11 methylenes, one at δ 23.0 (C-17), eight CH_2 at δ 29.5 (C-8 to C-15) and two at δ 31.5 (C-7, C-16). The assignments are based on DEPT, HMQC and comparison with related partial structures [6, 10].

Piperine (**2**) was obtained as the main constituent (yield 0.5% on the weight of dried fruits) by recrystallization (*vide* Experimental). The physical data of **2** were in good agreement with those reported [6, 11]. It should be noted that this is the first report of the isolation of **1** and its detailed spectral data. This compound was earlier detected in a mixture of amides [12].

EXPERIMENTAL

General. ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz): CDCl_3 with the spectra referenced to residual

Table 2. ^1H - ^1H relationship of pipericine (1) as observed in the COSY-45 spectrum

H	δ_{H}	Correlated protons with H	δ_{H}
2	5.70	3	7.10
3	7.10	2	5.70
		4	6.08
5	5.95	4	6.08
		6a	2.13
		6b	2.32
19	3.15	20	1.79

solvent signals. EIMS and HREIMS (probe): 70 eV. TLC: silica gel GF₂₅₄. Flash CC: silica gel 9385.

Extraction and isolation. Dried fruits (10 kg) of *P. nigrum* L. obtained from the local market of Karachi were crushed and extracted $\times 5$ with EtOH at room temp. After removal of solvent *in vacuo*, the syrupy residue was left overnight at room temp. when a white crystallize sepd out, which was filtered. On recrystallization from MeOH it formed fine needles (50.54 g), mp 128–129°, and was identified as piperine. The filtrate of piperine was partitioned between EtOAc and H_2O . The EtOAc phase was treated with a 4% aq. soln of Na_2CO_3 to remove acidic components. The constituents obtained on washing (H_2O), drying (Na_2SO_4), decolourizing (activated charcoal bed) and removal of solvent from the EtOAc layer was divided into petrol-soluble and petrol-insoluble frs. The petrol-insoluble fr. was subjected to flash CC using petrol, petrol-EtOAc, EtOAc, EtOAc-MeOH and MeOH (in order of increasing polarity). On mixing various eluates on the basis of TLC composition, a total of 19 frs were obtained, of which two were major (V and IX). V on TLC (CHCl_3 -MeOH, 19:1) afforded **1** in a pure state. Similarly IX on TLC with the same solvent system yielded further quantities of piperine.

Pipericine (1). Oily (3.8 mg). IR ν_{max} , CHCl_3 : 3300, 3100 (NH), 2900, 2850 (CH), 1654 (C=O), 1625 (C=C). UV λ_{max} EtOH: 256.8 nm. HREI-MS m/z (rel. int.): 335.3179 [M]⁺ (calcd for $\text{C}_{22}\text{H}_{41}\text{NO}$ m/z 335.3179) (23), 320.2914 ($\text{C}_{21}\text{H}_{38}\text{ON}$, [$\text{M}-\text{Me}$]⁺) (4.97), 263.2352 ($\text{C}_{18}\text{H}_{31}\text{O}$) (20), 235.2042 ($\text{C}_{16}\text{H}_{27}\text{O}$) (4), 166.1254 ($\text{C}_{10}\text{H}_{14}\text{NO}$) (4.5), 154.1235 ($\text{C}_9\text{H}_{16}\text{NO}$) (4), 152.1004 ($\text{C}_9\text{H}_{14}\text{NO}$) (15), 98.0723 (C_6H_{10}) (4), 97.0876 ($\text{C}_6\text{H}_{11}\text{N}$) (6), 85.0965 ($\text{C}_5\text{H}_{11}\text{N}$) (5), 71.0827 (C_5H_{11}) (5). EIMS m/z (rel. int.): 335 (15), 320 (7), 276 (6), 263 (18), 250 (4), 237 (6), 224 (3), 211 (4), 194 (6), 167 (12), 152 (38), 141 (10), 127 (22), 113 (35), 85 (33), 71 (48), 57 (100). ^1H NMR: Table 1. ^{13}C NMR: Table 1. COSY: Table 2.

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