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*ISO*BUTYLAMIDES FROM *PIPER RIDLEYI*

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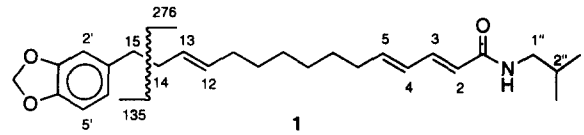
Key Word Index—*Piper ridleyi*; Piperaceae; *N-iso*butyl-15-(3′,4′-methylenedioxyphenyl)-2*E*,4*E*,12*E*-pentadecatrienamide; ridleyamide; retrofractamide.

Abstract—Extraction of the stems and leaves of *Piper ridleyi* with ether afforded *N-iso*butyl-15-(3′,4′-methylenedioxyphenyl)-2*E*,4*E*,12*E*-pentadecatrienamide (ridleyamide), as well as the known amide, retrofractamide, and sterols.

INTRODUCTION

A variety of unsaturated *iso*butylamides have been isolated from plant sources, in particular from members of the genus *Piper* [1]. These compounds are of interest because of their physiological activity and insecticidal properties [2]. Presently, we are investigating the constituents of Malaysian *Piper* species as part of a research programme seeking potent insecticides. We report herein the isolation and structure elucidation of a new *iso*butylamide, named ridleyamide **1**.



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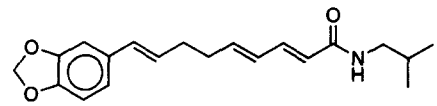

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RESULTS AND DISCUSSION

The dried stems and leaves of *P. ridleyi* were extracted with ether. Multiple column chromatography of the extract over silica gel led to the isolation of a sterol fraction, and two discrete compounds, **1** and **2**. GC–mass spectrometric analysis of the sterol fraction confirmed it to be a mixture of ergosta-5-en-3-ol, stigma-5,22-dien-3-ol and stigmasta-5-en-3-ol.

Compound **1** was obtained as a white crystalline solid, mp 95–98°. It exhibited a molecular formula of $C_{26}H_{37}NO_3$ ($[M]^+$ at *m/z* 411.2773) as determined by EI mass spectrometry and its IR spectrum contained absorptions due to NH (3302 cm^{-1}), dienamide (1657, 1626 and 1613 cm^{-1}) and methylenedioxy (1252, 1042 and 922 cm^{-1}) functional groups. The UV spectrum exhibited maxima at 206 and 261 nm ($\log \varepsilon$ 4.22 and 4.43, respectively). The ^{13}C NMR spectrum (Table 1) of **1** showed the presence of 26 carbons. In the ^1H NMR spectrum, signals attributed to an *N-iso*butyl moiety were observed at δ0.92 (6H, *d*, J = 6.7 Hz), 1.80 (1H, *m*), 3.16 (2H, *t*, J = 6.5 Hz) and 5.44 (1H, *m*). The ^1H NMR spectrum of **1** contained signals for a 3,4-methylenedioxyphenyl group and, furthermore, contained signals for six olefinic protons. Two of the olefinic signals appeared at δ5.74 (1H, *d*, J = 14.9 Hz) and 7.19 (1H, *dd*, J = 14.9 Hz and 9.4 Hz) and were attributed to protons H-2 and H-3, respectively, of an α,β-unsaturated carbonyl system. Signals were also observed at δ6.12 (1H, *dd*, J = 15.1 Hz and 10.5 Hz) and δ6.06 (1H, *dt*, J = 15.1 Hz and 6.6 Hz) and were attributed to the protons in a *trans*-relationship on a second bond that was conjugated to the first. The assignments of these protons were established by ^1H–^1H-COSY. The presence of an *iso*butylamide grouping was supported in the mass spectrum by a fragment ion at *m/z* 368 ($[M - CH(CH_3)_2]^+$, 5%). The remaining two olefinic proton signals resided together as a narrow multiplet at δ5.41. The position of these olefinic protons at positions γ and δ from the aromatic ring in **1** became evident when in the ^1H–^1H COSY spectrum, the signal at δ5.41 showed strong correlation with the multiplet signal for the methylene protons at C-14 (δ2.25, 2H), which, in turn, correlated with the triplet signal for the benzylic protons at C-15 (δ2.58, 2H). There was also a strong correlation between the signal at δ5.41 and another methylene proton signal at δ1.96 (2H, *m*), which supported the positioning of the double bond in isolation from other units of

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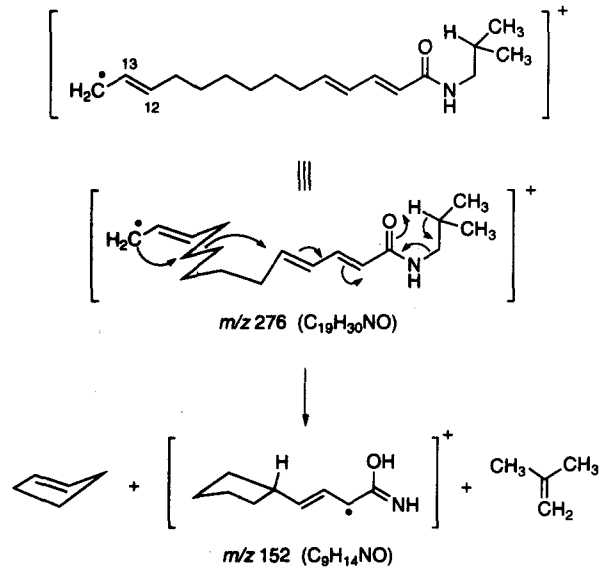
Table 1. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125.6 MHz, $CDCl_3$) of 1

Carbon	^{13}C NMR δ (ppm)	^{1}H NMR δ (ppm)	Multiplicity (J in Hz)	^{1}H–^{1}H-COSY correlation with
1	166.4			
2	121.8	5.74	*d* (14.9)	H-3
3	143.2	7.19	*dd* (14.9, 9.4)	H-2, H-4
4	128.2	6.12	*dd* (15.1, 10.5)	H-3, H-5
5	141.4	6.06	*dt* (15.1, 6.6)	H-4, H-6
6	33.0	2.14	*m*	H-5, H-7
7	28.8–29.4	1.36–1.48	*m*	H-6, H-8
8–10	28.8–29.4	1.22–1.35	*m*	H-7, H-11
11	32.5	1.96	*m*	H-10, H-12
12	129.3	5.41	*dt* (15, 7)	H-11, H-14
13	131.2	5.41	*dt* (15, 7)	H-11, H-14
14	34.7	2.25	*m*	H-13, H-15
15	35.9	2.58	*m*	H-14
1′	136.1	—	—	—
2′	108.1	6.67	*d* (1.3)	—
3′	147.5	—	—	—
4′	145.5	—	—	—
5′	109.0	6.62	*dd* (8.0, 1.7)	—
6′	121.2	6.72	*d* (7.8)	—
1″	47.0	3.16	*t* (6.5)	H-2″, NH
2″	28.7	1.80	*9 line multiplet* (6.7)	H-1″, H-3″, H-4″
3″, 4″	20.2	0.92	*d* (6.7)	H-2″
O_2CH_2	100.7	5.91	*s*	—
NH		5.44	*m*	H-1″

unsaturation. The position of the third double bond at C-12–C-13 in **1** was finally supported by the appearance in the mass spectrum of complementary fragment ions at m/z 276.2330 $[C_{18}H_{30}NO]^+$ and 135.0441 $[C_8H_7O_2]^+$. Another significant ion at m/z 152.1076 ($[C_9H_{14}NO]^+$, 10%) could be explained by subsequent fragmentation of the m/z 276 ion through a McLafferty rearrangement with expulsion of *iso*butylene and concomitant loss of cyclopentene, driven by participation of the dienamide function (Scheme 1).

The configuration of the isolated double bond could not be assigned easily because of the almost perfect superposition of the proton signals in the ^{1}H NMR spectrum. Fortunately, the C-12 and C-13 nuclei resonated separately (δ129.3 and 131.2, respectively). Their identity was confirmed through conventional HMQC and HMBC experiments. The vicinal proton couplings associated with each signal was measured using a single nuclei modulated quantum filtered COSY experiment. The spectrum was obtained using a ^{13}C-coupled homonuclear single quantum coherence (HSQC) sequence [3] with a BIRD sequence for extra suppression of ^{12}C bound protons [4]. The two olefinic proton signals resonated as doublet of triplets ($^3J_{H-H}$ 15, 7 Hz) (Fig. 1(b)) with the magnitude of the larger coupling providing clear evidence that the protons were *trans* and that the C-12–C-13 double bond was of (*E*)-geometry.

Based on the spectroscopic data, ridleyamide **1**, was an isomer of brachystamide B, a compound which was reported from *P. brachystachyum* [5]. Hence, **1** was assigned as *N-iso*butyl-15-(3′,4′-methylenedioxyphenyl)-2*E*,4*E*,12*E*-pentadecatrienamide.



Scheme 1. Proposed fragmentation pathway of daughter ion m/z 276 from compound **1**.

The other *iso*butylamide was identified as retrofractamide A **2** from its spectroscopic data. Retrofractamide A, a potent insecticide was isolated from *P. retrofractum* and synthesized by Banerji and coworkers [6].

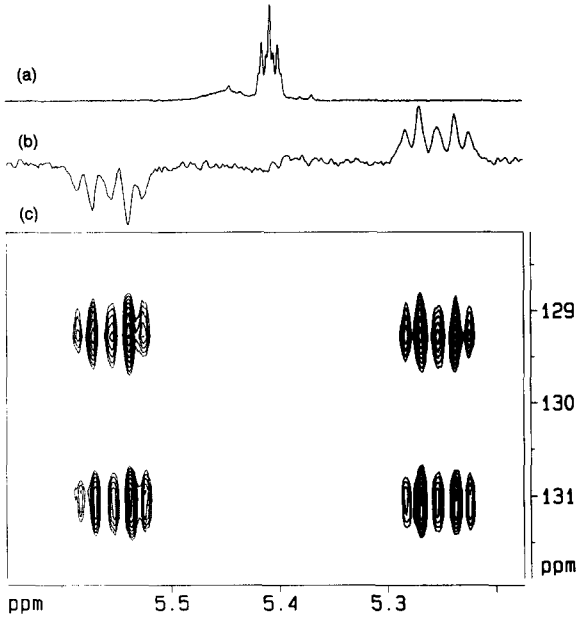


Fig. 1. Paritial NMR spectra of compound **1**. (a) Expansion of one-dimensional ^1H spectrum in the region δ5.2–5.6 showing second order pattern for H-12 and H-13 due to both protons. (b) ^{13}C Satellite subspectrum of one of the *trans*-protons from the ^{13}C-coupled HSQC experiment. (c) Contour plot of two-dimensional sub-spectrum from the ^{13}C-coupled HSQC experiment (dotted lines refer to negative contours).

EXPERIMENTAL

General. Mps: uncorr. IR: KBr pellets. UV: MeOH. ^1H and ^{13}C NMR: $CDCl_3$. MS: 70 eV. CC: silica gel G (70–230 mesh).

Plant material. Stems and leaves of *P. ridleyi* C. DC were collected from Sungai Long Intake 1, Jeli Kelantan, Malaysia. The species was identified by Mr A. Z. Ibrahim (Universiti Kebangsaan Malaysia) and a voucher specimen (ALM 3738) is deposited in the herbarium of the Botany Department, Universiti Kebangsaan Malaysia, (Bangi, Selangor, Malaysia).

Extraction and isolation. Air-dried stems and leaves (366.2 g) were extracted twice with Et_2O at room temp. The Et_2O extract was filtered and evapd to give a gum (2.4 g). Chromatographic separations of the crude extract with Et_2O–petrol as eluent afforded sterols (37 mg), ridleyamide **1** (540 mg) (see Results and Discussion for data) and retrofractamide A **2** (12.3 mg).

Retrofractamide A (**2**). Pale yellow amorphous solid, mp 133–138°. IR λ_{max}: 208 and 263 nm; ν_{max}: 3302, 1657, 1630, 1615, 1549, 1505, 1491, 1256, 1044, 999 cm^{-1}. ^1H NMR (500 MHz): δ0.92 (6H, *d*, J = 6.7 Hz, $CH(CH_3)_2$), 1.80 (1H, *9 line multiplet*, J = 6.7 Hz, $CH(CH_3)_2$), 2.31 (4H, *m*, $-CH_2-$), 3.16 (2H, *dd*, J = 6.5 Hz and 6.5 Hz, $-CH_2-N-$), 5.47 (1H, *br t*, J = 5.3 Hz, –NH), 5.76 (1H, *d*, J = 15.0 Hz, –CH = CH–CO), 5.94 (2H, *s*, $O-CH_2-O$), 6.02 (1H, *dt*, J = 15.7 Hz and 6.7 Hz, –CH = CH–CH_2–), 6.09 (1H, *dt*, J = 15.1 Hz and 6.5 Hz, $-CH_2-CH = CH-$), 6.17 (1H, *dd*, J = 15.1 Hz and 10.5 Hz, $-CH = CH-$), 6.31 (1H, *d*, J = 15.6 Hz, Ar-CH = CH–), 6.73 (1H, *d*, J = 7.9 Hz, Ar-H), 6.75 (1H, *dd*, J = 8.0 Hz and 1.4 Hz, Ar-H), 6.88 (1H, *d*, J = 1.4 Hz, Ar-H), 7.19 (1H, *dd*, J = 15.0 Hz and 10.5 Hz, –CH = CH–CO). MS *m/z* (rel. int.): 327 ($[M]^+$, 9), 161 (67), 131 (100), 103 (30), 77 (10). Spectral data (^1H NMR, EIMS, UV, IR) similar to published data [7].

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