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Note

Lipid derivatives from *Mucuna pruriens* seeds

Laxminarain Misra* & Hildebert Wagner[$]

*Phytochemistry Division, Central Institute of Medicinal and Aromatic Plants, Lucknow 226 015, India

[$]Department of Pharmacy, Center for Pharma Research, Butenandtstrasse 5-7, University of Munich, 81377 Munich, Germany

E-mail: laxmisra@hotmail.com

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The seeds of *Mucuna pruriens* have afforded three new lipid derivatives from its *n*-hexane extract, namely:*(Z)*-Triacont-5,7,9-triene; *(Z)*-Docos-2,4,6-trien-1,8-diol and *(Z)*-Docos-5-en-1-oic acid. Their structures have been elucidated mainly by spectroscopic methods.

Keywords: *Mucuna pruriens* (L.) Papilionaceae, seeds, Lipid derivatives, L-DOPA, fatty acid

IPC: **Int.Cl.**[7] **C 07 C**

Mucuna pruriens (l.) DC is a medicinal plant of India well known for its use as a remedy for male impotency, as an aphrodisiac as well as a cure for sexual debility and as a nervine tonic. The pods are known to have anthelmintic and the seeds anti-inflammatory and aphrodisiac properties[1]. The seed powder has shown anti-Parkinson activity which is supposed to be due to the presence of L-DOPA in it. The presence of tetrahydroisoquinoline alkaloids from its seeds has recently been reported[2,3]. Work on the non-polar part of the extract has yielded some lipid derivatives whose structure elucidation by spectroscopic methods is being reported now.

Results and Discussion

Fatty acids. The seeds were extracted with *n*-hexane, acetone and ethyl acetate. The yield of oil was: *n*-hexane (4.1%), acetone (4.5%) and ethyl acetate (3.8%). The main components of the oil were the palmitic, stearic, oleic and linoleic acids[4] (**Table I**).

Compounds 1-3. From the *n*-propanol extract, three compounds were purified and their structure elucidated by spectroscopic methods. The IR of compound **1** showed bands at 2950, 1645, 1440,1385 and 720 cm^{-1} indicating that it was a hydrocarbon. The EIMS showed $[M]^+$ ion at *m/z* 416 and HRMS at *m/z* 416.7721 for $C_{30}H_{56}$. The ^1H NMR spectrum showed typical signals for $CH_3(CH_2)_n$ at δ 0.89, 1.28 and 1.59. Additional signals (overlapping triple doublets, *J*= 11.0, 5.5 Hz, 6H) at δ 5.33 followed by multiplets at δ 2.05 and 2.28 (each 2H) indicated that **1** contains three conjugated double bonds. The lower *J* value (11.0 Hz) of double bond protons clearly indicated that they belong to the *Z* form. The presence of three conjugated double bonds was further supported by the typical signals in ^{13}C NMR (**Table II**). The prominent fragments in MS of **1** at *m/z* 359 and 373 as well as 281 and 267 due to α and β fission of double bonds, further established that double bonds were situated at C-5, C-7 and C-9. Thus the structure of compound **1** was given as (*Z*)-triacont-5,7,9-triene.

Similarly, compound **2** showed IR bands at 3400-3300, 2950, 1640, 1440, 1380 and 720 cm^{-1} indicating the presence of OH functional group. Its EIMS showed $[M]^+$ at *m/z* 336 and HRMS at *m/z* 336.5562 for $C_{22}H_{40}O_2$. Its ^1H NMR spectrum clearly indicated the presence of $CH_3(CH_2)_n$ by the typical signals at δ 0.88, 1.28 and 1.61. Additional signals (overlapping

Table I — Extraction and identification of fatty oil from *M. pruriens* seeds by GC-MS

Extract No	Name of solvent	Solvent (mL)	Material (g)	Yield of oil (g)	% of oil	Main fatty acid
MHX-1299	*n*-Hexane	2×40	20	0.82	4.1	Palmitic, Stearic, oleic, linoleic
MAC-1299	Acetone	2×40	20	0.89	4.5	Palmitic, oleic, linoleic
METAC-0200	Ethyl acetate	2×40	20	0.76	3.8	Palmitic, oleic, linoleic

Table II — ^{13}C NMR spectral data of compound **1-3** in CD_3OD

Carbon No.	**1**	**2**	**2a**	**3**	**3a**
1	14.11	62.01	62.05	179.47	179.42
2	22.72	37.50	37.52	37.93	38.01
3	31.93	125.22*	125.34*	28.05	28.05
4	35.22	137.11*	137.92*	34.11	34.14
5	128.11*	130.72*	130.72*	128.91*	128.81*
6	130.01*	130.92*	130.92*	130.79*	130.53*
7	130.04*	128.13*	128.22*	34.13	34.25
8	130.20*	149.32*	149.44*	29.66**	29.66**
9	130.15*	34.05	34.30	29.62**	29.68**
10	128.00*	76.13	77.03	29.62**	29.68**
11	34.01	34.00	34.04	29.62**	29.68**
12-19	29.88-29.7**	29.34-24.91**	29.55-24.80**	29.62-25.00**	29.68-25.02**
20	29.36**	31.91	32.02	32.52	31.92
21	29.36**	22.69	23.11	23.62	22.94
22	29.46**	14.12	14.02	14.41	14.40
23	28.88**	-	-	-	-
24	24.00**	-	-	-	-
25	24.81**	-	-	-	-
26	25.83**	-	-	-	-
27	27.01**	-	-	-	-
28	31.92	-	-	-	-
29	22.73	-	-	-	-
30	14.11	-	-	-	-
OMe	-	-	-	-	51.12
OAc	-	-	20.72, 20.78	-	-

*, ** Values interchangeable within the same column

triple doublets, *J*= 11.0, 5.5 Hz, 6H) at δ 5.34 followed by the multiplets at δ 2.04 and 2.30 (each 2H) indicated that **2** contains three conjugated double bonds. The lower *J* value (11.0 Hz) of double bond protons clearly indicated that they belong to the *Z* form. The presence of three conjugated double bonds was further supported by the typical signals in ^{13}C NMR (**Table II**). The prominent fragments in MS of **2** at *m/z* 291 and 305 as well as 213 and 199 due to α and β fission of double bonds, further established that double bonds were situated at C-2, C-4 and C-6. The unsaturation at C-2 was further supported by the presence of two doublets for H-1 at δ 4.17 and 4.32 (*J*= 10 and 6 Hz) for CH_2OH at C-1 which got shifted to 4.90 and 4.82, respectively, after its acetylation into **2a**. The presence of another multiplet at δ 3.82 which also got shifted to 4.45 after acetylation, confirmed the presence of another hydroxyl group in the molecule. ^{13}C NMR of **2** showed a triplet at δ 34.05 for C-8 and a doublet at 77.0 for C-9 confirming that the hydroxylation is at C-9 and not C-8 which was also supported by the data of ^{13}C NMR of **2a** (**Table II**). However, the unusual deshielding of C-7 could be due to the neighbouring hydroxyl group. Thus with these data, the structure of compound **2** was elucidated as (*Z*)-docos-2,4,6-triene-1,8-diol.

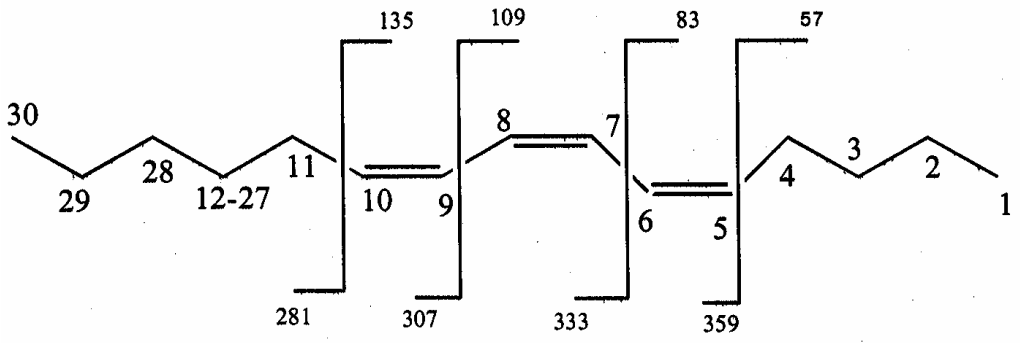
Compound **3** showed IR bands at 3400-3100, 1720 (COOH), 2950, 1640, 1440, 1380 and 720 cm^{-1} and $[M]^+$ at *m/z* 338 and HRMS at *m/z* 338.5728 for $C_{22}H_{42}O_2$ in its MS spectrum. Its ^{1}H NMR spectrum indicated the presence of $CH_3(CH_2)_n$ by the typical signals at δ 0.89, 1.28 and 1.61. Additional signals (overlapping triple doublets, *J*= 11.0, 5.5 Hz, 2H) at δ 5.33 alongwith the multiplets at δ 2.06 and 2.19 (each 2H) clearly indicated that **3** contains a double bond. The lower *J* value (11.0 Hz) of double bond protons clearly indicated that they are in *Z* form. The presence of the double bond was further supported by the typical signals in ^{13}C NMR (**Table II**). The prominent fragments in MS of **3** at *m/z* 251 and 265 as well as 225 and 211 due to α and β fission of double bonds, further established that the double bond was situated at C-5 (**Scheme I**). The presence of terminal carboxylic group at C-1 was supported by the signals at δ 179.47 and 37.93 (for C-2) in ^{13}C NMR. The presence of the carboxylic group was again supported by the methylation of **3** into **3a** giving supporting signals in ^{1}H and ^{13}C NMR spectra of **3a** confirming the structure of **3** as (*Z*)-docos-5-en-1-oic acid.

The structures of lipid derivatives **1-3** show that besides normal fatty acids and unsaturated fatty acids, hydroxylated unsaturated lipids are also present in the extract which could be of biological significance. On the other hand, the isolation of the unsaturated straight chain hydrocarbon is quite unusual.

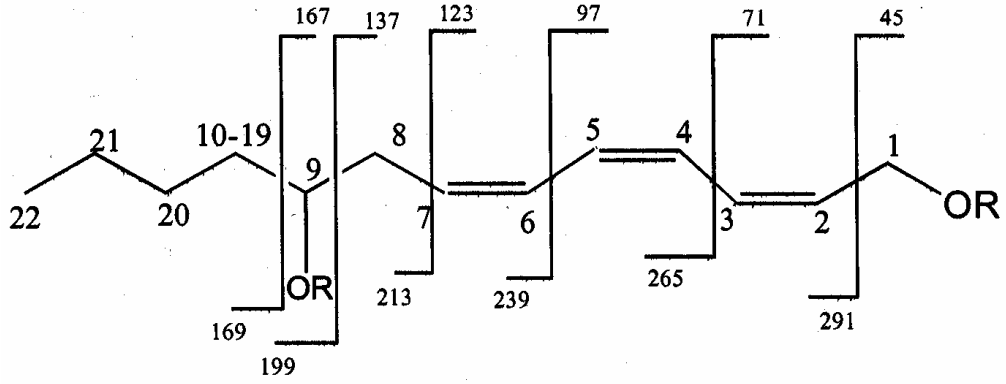
Experimental Section

^{1}H and ^{13}C NMR, DEPT spectra were recorded on Jeol instrument at 400 MHz and 100 MHz, respectively. IR was recorded on a Perkin-Elmer 1710 instrument and HRMS as well as EIMS was recorded on Jeol JMS 700 at 70 eV. GC-MS of the esters were carried out on Perkin-Elmer, Turbo Mass Auto XL instrument.

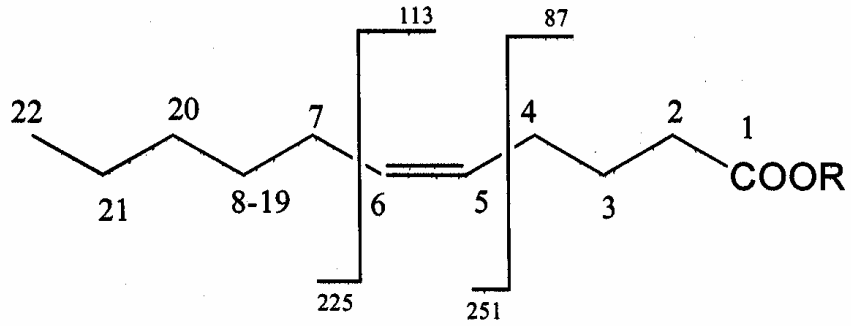
Extraction of lipid derivatives. The residual part of the seeds (500 g), obtained after the extraction by acetone, was further extracted by *n*-propanol to ascertain the presence of polar fatty acid (lipid)



1 (MS fragments in m/z)



2, R= H (MS fragments in m/z)
2a, R= Ac



3, R= H (MS fragments in m/z)
3a, R= Me

Scheme I

derivatives. The seed powder was extracted by 1.25 L *n*-propanol (three times) by shaking overnight at rt to obtain 8.84 g extract. Out of this extract, 2.5 g was subjected to Medium Pressure Liquid Chromatography (Gilson instrument model 303) at a pressure of 500 psi and flow rate 2.5 mL/ min over Buechi glass column on silica gel (230-400 mesh), with mobile phase as *n*-butanol-*n*-propanol-water, 6:6:1). The MPLC gave mainly 5 fractions of which the first two were a complex mixture of fatty acid derivatives and the third contained mainly sucrose (170 mg). The 4th fraction yielded some basic material while the 5th fraction yielded L-DOPA (10 mg). The first fraction on crystallization gave compound **1** (750 mg, R_f 0.90, TLC, *n*-butanol-*n*-propanol, 1:1) while the second fraction on crystallization gave compound **2** (40 mg, R_f 0.70, TLC, *n*-butanol-*n*-propanol-water, 4:4:1) and its mother liquor after centrifugation and TLC gave compound **3** (30 mg, R_f 0.60, TLC, *n*-butanol-*n*-propanol-water, 4:4:1) and a complex mixture of further neutral compounds.

***(Z)*-Triacont-5,7,9-triene 1**: Viscous oil; IR ($CHCl_3$): 2950, 1645, 1440, 1385 and 720 cm^{-1}; HRMS (m/z): 416.7721 (calcd. for $C_{30}H_{56}$: 416.7724); EIMS (m/z, relative intensity): 416 (25) $[M]^+$, 373 (36) $[M-43]^+$, 359 (51) $[M-57]^+$, 333 (42) $[M-83]^+$, 307 (58) $[M-109]^+$, 281 (50) $[M-135]^+$, 267 (60) $[M-149]^+$, 149 (50), 135 (100), 109 (35), 83 (60), 57 (60), 43 (55); ^1H NMR (CD_3OD): δ 0.89 (6H, overlapping t (*J*= 6.5 Hz), H-1 and H-30), 1.28 (36H, broad s, H-2 and H-13 to H-29), 1.59 (4H, m, H-3 and H-12), 2.05 and 2.28 (2H each, m, H-4 and H-11), 5.33 (6H, overlapping m, H-5 to H-10); ^{13}C NMR (CD_3OD): see **Table II**.

***(Z)*-Docos-2,4,6-trien-1,8-diol 2**: White crystals, m.p. 120°C; IR ($CHCl_3$): 3400-3300 (OH), 2950, 1640, 1440, 1380 and 720 cm^{-1}; HRMS (m/z): 336.5562 (calcd. for $C_{22}H_{40}O_2$: 336.5568); EIMS (m/z, relative intensity): 336 (8) $[M]^+$, 305 (16) $[M-CH_2OH]^+$, 291 (20) $[M-45]^+$, 265 (8) $[M-71]^+$, 239 (12) $[M-97]^+$, 213 ((10) $[M-123]^+$, 199 (10) $[M-137]^+$, 169 (20) $[M-167]^+$, 55 (100), 41 (80); ^1H NMR (CD_3OD): δ 0.88 (3H, t (*J*= 6.5 Hz), H-22), 1.28 (22H, s br, H-11 to H-21), 1.61 (2H, m, H-10), 2.04 and 2.30 (1H each, m, H-8), 3.82 (1H, m, H-9), 4.17 and 4.32 (1H each, dd (*J*= 10, 6, 3 Hz), H-1), 5.34 (6H, overlapping m, H-2 to H-7); ^{13}C NMR (CD_3OD): see **Table II**. Diacetate derivative **2a**: 20 mg **2** was taken in a flask and 1 mL acetic anhydride and 1 drop pyridine was added to it. After usual work-up **2a** was obtained (15 mg): ^1H NMR, ($CDCl_3$): δ 2.10, 2.11 (3H each, s, OAc), 4.45 (1H, m, H-9), 4.90 and 4.82 (1H each, dd (*J*= 10, 6, 3 Hz), H-1); ^{13}C NMR (CD_3OD): see **Table II**.

***(Z)*-Docos-5-en-1-oic acid 3**: Viscous liquid; IR ($CHCl_3$): 3400-3100 (COOH), 2950, 1720 (COOH), 1640, 1440, 1380 and 720 cm^{-1}; HRMS (m/z): 338.5728 (calcd. for $C_{22}H_{42}O_2$: 338.5726); EIMS (m/z, relative intensity): 338 (18) $[M]^+$, 293 (20) $[M-45]^+$, 279 (25) $[M-CH_2COOH]^+$, 265 (22) $[M-73]^+$, 251 (10) $[M-87]^+$, 225 (8) $[M-113]^+$, 211 (8) $[M-127]^+$, 159 (33), 117 (100), 113 (45), 87 (15); ^1H NMR (CD_3OD): δ 0.89 [3H, t (*J*= 6.5 Hz), H-22], 1.28 (24H, s br, H-9 to H-21), 1.61 (2H, m, H-8), 2.06 (2H, m, H-7), 2.19 (2H, m, H-4), 1.38 (2H, m, H-3), 2.77 (2H, m, H-2) 5.33 (2H, overlapping m, H-5 and H-6); ^{13}C NMR (CD_3OD): see **Table II**. Methyl ester **3a**: 20 mg **3** was taken in a flask and 2 mL freshly prepared diazo methane was added to it. After usual work-up **3a** was obtained (20 mg): ^1H NMR ($CDCl_3$): δ 3.66 (3H, s, OMe); ^{13}C NMR (CD_3OD): see **Table II**.

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