Pak J Sci Ind Res 1998 41 (1) 6-10

ISOLATION AND NMR-ASSIGNMENTS OF 19αH-LUPEOL FROM *E. HELIOSCOPIA* LINN *(N.O. EUPHORBIACEAE)*

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(Received 4 August 1995; accepted 18 August 1997)

A triterpenoid resembling lupeol was isolated from the latex of *Euphorbia helioscopia* Linn. The structure was elucidated by physico-chemical methods and confirmed as 19αH-lupeol.The generated data have been compared with those of stereoparent lupeol, nepehinol and the previously isolated compound which was erroneously given the same structure.

Key words: *Euphorbiaceae, Euphorbia helioscopia latex,* 19αH-Lupeol.

Introduction

Previously we isolated lupeol **(I)** from the epicuticular leaf wax of *Euphorbia helioscopia* (Nazir *et al.* 1977, 1983, 1993). Another triterpenoid has now been isolated from the latex of the species. That triterpenoid has a lower m.p. and is less polar than lupeol in argentation TLC. The IR spectrum resembles that of lupeol or nepehinol (Ahmad *et al.* 1985) **(II)**.The mass spectrum followed the lupeol pattern but with minor variations in the intensities of certain peaks. On the basis of the m.p., specific rotation and some spectroscopic data, the triterpenoid is thought to be the one isolated from *Macluria pomifera* and designated as 19αH-lupeol (III). However, as our NMR data do not fully resemble with the reported one (Gearien and klein 1975), it is deemed necessary to compare our data from the isolated triterpenoid with the chemical structure of19αH-lupeol. A satisfactory correlation would then confirm its first isolation from the natural source of interest as the existence of such compounds, due to their spatial interaction, is rare (Budesinsky and Vystrcil 1970; Vystrcil and Budesinsky 1970).

Experimental

M.P.'s were recorded on a Fisher John M. P. apparatus without any correction. Optical rotation was recorded in chloroform on a POLAX-D polarimeter. The IR spectra were recorded as dispersion in KBr on an IR spectrophotometer (Hitachi 270-30). The NMR spectra were determined in deuterated chloroform solution with TMS as an internal standard using a 90 MHz spectrometer (Jeol EX 90) with a built-in program for DEPT and quaternary carbon atoms. The mass spectra were recorded on a CEC-21-110 B high resolution Mass Spectrometer using the Finnigon-Incos data and the Jeol Mass Spectrometer JMS-AX 505H.

Recovery of the Resin: Mature plants of *E.helioscopia* were collected from the laboratory campus during the last week of Feb. The stems were given an incision and the oozing latex was dropped into ethyl acetate to freeze the enzymatic action and dissolve the resin. The fibrous material and the water of the plant which formed the lower layer were removed. The organic layer was dried over anhydrous sodium sulphate and the solvent was removed.

Separation of Alcoholic Fraction: The resinous mass obtained above (2.5g)was charged to silica gel plates (20x 20x0.1cm, 25 Nos.) and developed in toluene. The plates were sprayed with 2,7-dichlorofluorescein. Under U V light, the zones were marked, scratched, and extracted with chloroform to give 1.0g (40%yield) of the alcoholic latex component.

Acetylation of the Alcoholic Fraction: The alcoholic fraction (1.0 g), acetic anhydride (10ml), and pyridine (2 ml) were heated on a water bath for two h. The reaction mixture was poured into ice-water. The acetylated product was extracted with ether, washed with water followed by removal of solvent to give 1.0 g of the corresponding acetates.

Isolation of 19αH-Lupeol (III): The acetylated fraction was charged to silica gel plates impregnated with silver nitrate (90:10) (20x20x0.1 cm, 12 Nos.) and developed three times with hexane-dichloromethane (3:2) as eluant. After repeating the process described above, the zone with R 0.5

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was obtained (75mg, 7.5% of resin). This material was crystallised out from boiling methanol to give needles with m.p. 202-204°, $[\alpha]^{D}_{33}$ +15.94° (C 0.0037,$CHCl_3$). The IR spectrum in KBr showed peaks at 2952, 1728, 1650, 1258 and 890 cm^{-1}. Table 1 shows signals in ^{1}HNMR. Mass fragmentations m/e (%) are listed as follows: 468.3947 (16); 409.3777 (16); 408.3759 (49); 393.3524 (19); 367.3282 (1); 366.3240 (15); 365.3215 (56); 339.3048 (12); 298.2640 (14); 297.2561 (28); 249.1818 (13); 229.1959 (23); 218.2025 (17); 205.1952 (18); 204.1867 (22); 203.1794 (35); 191.1795 (32); 190.1708 (42); 189.1644 (100); 188.1546 (10); 187.1477 (19);175.1493 (25); 163.1487 (11); 162.1394 (10); 161.1331 (31); 149.1318 (20); 148.1227 (13); 147.1165 (28); 137.1324 (12); 136.1236 (30); 135.1173 (60); 134.1083 (22); 133.1008 (26); 123.1170 (27); 122.1083 (20); 121.1106 (58); 120.0928 (13); 119.0860 (32); 109.1026 (60); 108.0939 (26); 107.0868 (51); 106.0780 (8); 105.0713 (28).

The acetate (60 mg),0.5 M ethanolic potassium hydroxide (20 ml) and benzene (5 ml) were refluxed on a water bath for two h. After adding water, the reaction mixture was extracted with ether. Further work up yielded 19αH-lupeol (55 mg 100%) which was crystallised out from methanol-dichloromethane at room temperature to give long needles m.p.182-3°, $[\alpha]^{D}_{30}$+11.71°. IR spectrum in KBr showed absorption peaks at 3445, 2975, 1660, 1468, 1385, 1050 and 900 cm^{-1}. For ^{1}HMNR see Table 1. Mass fragmentation m/e (%) showed as follows:

428 (77); 218 (64); 207 (100); 189 (59); 175 (25); 147 (34); 135 (56); 121 (48); 109 (79); 108 (65); 107 (59); 95 (73);81 (53); 69 (47); 55 (31).

Oxidation to 19αH-lupenone: To a stirred, cooled solution of 19αH-lupeol (18 mg) in dry acetone (50 ml), Jones reagent [Fieser and Fiesir 1968] (10 ml) was added portionwise.The excess reagent was destroyed with methanol and the oxidised product was extracted with ether (15 mg, 83%), m.p.180-182°, IR in KBr: 2952, 2860, 1710, 1458, 1384, 894 cm^{-1}. For the ^{1}HNMR see Table 1. Mass fragmentation m/e(%) showed as follows:

424 (82); 409 (21); 381 (7); 368 (11); 342 (9); 313 (32); 300 (8); 271 (4); 245 (24); 232 (10); 218 (32); 205(100); 189 (32); 177 (18); 161 (21); 149 (26); 121 (35); 109 (55); 95 (47); 81 (32); 69 (23); 55 (21).

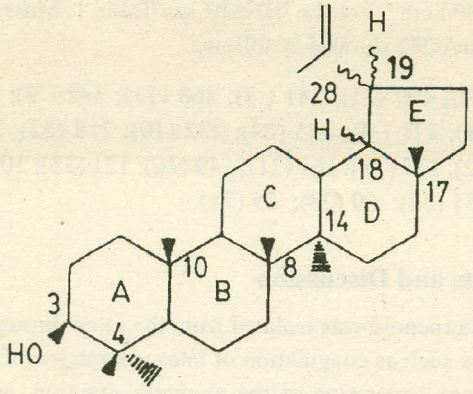
Results and Discussion

The triterpenoid was isolated from the latex through a series of steps such as coagulation of latex, extraction of the resinous mass, separation of the alcoholic fraction, and finally by argentation PLC, as the acetate derivative with a yield of 7.5% on the basis of resinous mass. When crystallised out from boiling methanol, needles with m.p. 202-4° were obtained. The IR spectrum showed the ester carbonyl (1728, 1258, cm^{-1}) and an exocyclic methylene group (1650 and 890 cm^{-1}). The high resolution mass spectrum showed parent peak at 468.3947 AMU which corresponds to the elemental composition of 19αH-Lupeol acetate $C_{32}H_{52}O_2$ (calculated 468.3970). The base peak was at 189.1644 AMU which was the characteristic peak of lupene or hopene-like structures. The fragmentation pattern was analogous to lupeol acetate but with minor variation in the intensities of certain peaks. The acetate was saponified to give the triterpenoid, m.p. 182-3°. The IR spectrum showed broad bands centered at 3445 and 1050 (OH),2975, 2895,1468,1395 (C-H),1660 and 900 cm^{-1} (exocyclic methylene). All the peaks was present in the IR of lupeol and nepehinol. The mass spectrum showed m/e 426 (77.27), with a base peak at 207. The IR spectrum of the ketone obained from the oxidation of the alcohol showed the keto group (1710) and the exocyclic methylene group (1650 and 894 cm^{-1}). The mass spectrum showed m/e 424 (82.13) with a base peak at 205.The fragmentation of the acetate, the alcohol and the ketone were well explained assuming the compound to be an epimer of lupeol (Fig.1). A likely variation of the structure could be the configuration of

Table 1

^{1}HNMR data of triterpenoids

Compound	C-23	C-24	C-25	C-26	C-27	C-28	C-29	C-30	C-19
Lupeol[4]	0.94	0.76	0.83	1.03	0.96	0.79	4.57 & 4.68	1.67	2.38 (dd)
Lupeol Acetate[4]	0.84	0.84	0.84	1.04	0.97	0.97	//	1.68	
Nepehinol[4]	0.90	0.76	0.83	1.04	0.96	0.90	4.62 & 4.70	1.68	2.50 (dd)
Nepehinol Acetate[4]	0.84	0.86	0.84	1.04	0.90	0.90	//	1.68	
19αH-Lupeol Acetate[5]	0.84	0.84	0.84	1.027	0.929	0.78	4.46 & 4.60	1.68	
19αH-Lupeol	0.91	0.76	0.84	1.04	0.96	0.91		1.68	2.54 (dd)
19αH-Lupeol Acetate	0.87	0.84	0.84	1.05	0.91	0.91		1.68	
19αH-Lupenone	1.09	0.93	1.08	1.04	0.93	0.93		1.68	



the hydroxyl group at position 3. The proton at that carbon atom appeared as a broad multiplet (δ 3.16-3.24) which can easily be confirmed as axial.It may be added here that the equatorial carbinylic proton at C-3 in triterpenes of the lupane series with 3α OH group absorbed near δ 3.40 (Proietti *et al.* 1981; Monaco and Previtera 1984). This signal was generally much narrower because only one axial-equatorial and one equatorial- equatorial coupling (J=2.5 Hz) was visible and there was no larger axial- axial coupling (Das 1971). The β oriented OH at position 3 was also confirmed by its oxidation when shifting of the methy1 groups at C-4 and C-10 in the NMR of 3-keto-compound was evident. (see Table 2).

Table 2

^{13}C-NMR assignments of triterpenoids

Carbon No.	Lupeol[12]	Nepehinol[1]	19αH-Lupeol	19αH-Lupeol acetate	19αH-Lupeol ether[15]	19αH-Lupenone	Lupenone
1	38.7	36.87	38.83(t)	38.53	38.9	39.62	39.5
2	27.4	27.55	27.50(t)	23.78	22.5	33.38	34.5
3	78.8	78.97	79.04(d)	81.00	88.9	217.83	247.9
4	38.8	38.75	38.93(s)	37.88	39.0	47.31	47.2
5	55.2	55.38	55.46(d)	55.57	56.2	54.98	54.8
6	18.3	18.35	18.44(t)	18.31	18.5	19.66	19.6
7	34.2	34.15	34.19(t)	34.19	34.4	34.10	33.5
8	40.8	40.95	41.04(d)	41.07	41.2	40.87	40.7
9	50.4	50.63	50.71(d)	50.68	50.9	50.51	49.7
10	37.1	37.21	37.30(s)	37.23	3 7.5	36.90	36.8
11	20.9	20.84	20.93(t)	20.95	21.1	20.97	21.4
12	25.1	25.16	25.26(t)	25.22	25.4	25.15	25.1
13	38.0	34.81	3 4.91(d)	34.91	35.1	34.90	38.1
14	42.8	41.79	41.88(s)	41.89	42.0	41.76	42.8
15	27.4	27.60	27.63(t)	22.63	27.8	27.50	27.4
16	35.5	30. 60	36.95(t)	36.95	37.1	36.75	35.4
17	42.9	43.02	42.11(s)	43.12	43.3	43.08	42.0
18	48.2	50.63	50.71(d)	50.68	50.9	49.91	48.2
19	47.9	44.76	44.86(d)	44 .85	45.0	44.69	47.8
20	150.6	150.47	150.51(s)	150.53	150.6	150.50	150.5
21	29.8	29.67	30.68(t)	30.70	30.9	30. 58	29.8
22	39.9	41.38	41.45(t)	41.46	41.6	41.32	39.9
23	28.9	27.98	28.05(q)	27.98	28.2	26.55	26.6
24	15.4	15.33	15.38(q)	16.50	16.3	21.33	21.0
25	16.1	16.08	16.14(q)	16.35	16.3	15.90	15.8
26	15.9	15.98	16.05(q)	16.02	16.3	15.69	15.9
27	1 4 . 5	14.37	14.45(q)	14.41	14.6	14.26	14.4
28	18.0	25.10	20.62(q)	20.62	20.8	20.52	18.0
29	109.2	108.92	108.97(q)	108.99	109.2	108.90	109. 2
30	19.3	2 0.55	25.10(q)	25.10	25.3	25.06	19.3
31	—	—	—	17.90	57.6	—	—
32	—	—	—	21.27	—	—	—

The second likely variation in the structure could be the shifting of the methyl groups or the iso-propylindene group. On comparison of positions for the angular methy1 groups in the ^{1}HNMR spectrum with the stereoparent lupeol, reported data for the erroneously designated 19αH-lupeol (Gearien and Klein 1975), and nepehinol, the substituents on ring A, B and C fell at the same positions in all the three triterpenols but the angular group on ring E (C-28) lay at (δ 0.91, which was neither in agreement with lupeol, nor with previously isolated and erroneouses designated 19αH-lupeol (Geasien and klein 1975) but δ was close to nepehinol. Another variation in the structure could be the stereochemistry at C-19. In lupeol the hydrogen at C-19 is β (Ca.100%), with the isopropylidene group was slightly α and equatorial in nature. If the trans annelation of rings D and E was preserved, the cis orientation of the side chain at C-19 with respect to the 17β-methyl group seemed to be more disadvantageous than in lupeol or the hopane series. That may be assumed on the basis of attempts for the preparation of 19αH-lupeol derivatives. Only such 19αH-lupane derivatives were prepared by direct isomerisation in which the side chain at C-19 could be stabilized in the β-configuration by intramolecular reactions with the functional group at C-28 under formation of another cycle. The split pattern of the olefinic protons differed from lupeol. The proton cis to the methyl group appeared only as a broad signal whereas a split signal was observed in lupeol, meaning thereby only small coupling constants and smaller angles with the coupling protons. The isopropylidene group in lupeol was equatorial but it was more stable due to the absence of any interaction. The proton being in the axial configuration, was expected to appear downfield. Contrary to lupeol, the isopropylidene group in 19αH-lupeol was in the axial configuration and due to axial-axial interaction was less stable. The proton was equatorial and hence lay upfield. That was as observed as the proton in lupeol lay between δ 2.28 to δ 2.55 whereas in 19αH-lupeol it appeared at δ 2.49 to δ 2.59.

In the ^{13}CNMR, spectrum of 19αH-lupeol, 21 resonance lines fell at positions differing less than 0.2 ppm from lupeol. The appearance of a double intensity signal at δ 50.71 was absent in lupeol (Wenkert *et al.* 1978) but present in nepehinol (Ahmad *et al.* 1985). Looking at the signals of the quaternary carbon atoms (4,8,10,14,17), the position of C-14 and C-17 signals differed significantly, which indicated a configurational difference in ring E. The position of a carbon signal changes with the configurational interchange of the substituents. This was well demonstarted in the assignments for oleanane derivatives (Ricca *et al.* 1978). Accordingly, the signal for C-19 lay upfield compared with lupeol. The change in configuration also affected the signal for C-18 which shifted downwards as compared with lupeol. Considering the principal of structure additivity (Balogh *et al.* 1973) it was concluded that identical resonance pattrens must present an identical partial structure present in the analogues as well as the unknown; for example the rings A,B,C in the structure of lupeol and C,D,E and the substituent in 19αH-lupeol methyl ether (Pauptit *et al* 1984) led to the completion of these assignments (Table-3).

Table 3

Substituent in 19αH-lupeol methyl ether

Triterpenoid	Isopropylidene group	Configuration of		
		H-18	H-19	H-3
Lupeol I	α	α	β	α
Nepehinol II	β	β	α	α
19αH-Lupeol III	β	α	α	α
19αH-Lupeol Acetate	β	α	α	α
19αH -Lupenone	β	α	α	-

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