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SESQUITERPENE LACTONES FROM *ENHYDRA FLUCTUANS*

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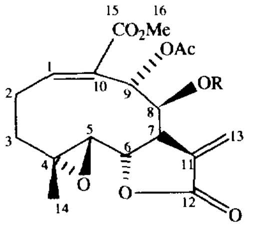
Key Word Index—*Enhydra fluctuans*; Compositae; sesquiterpene lactones; chlorine-containing melampolides.

Abstract—The extract of the leaves of *Enhydra fluctuans* gave, in addition to three known sesquiterpene lactones, two new chlorine containing melampolides. The structure and stereochemistry of the new compounds were elucidated by NMR techniques and chemical reactions.

INTRODUCTION

Enhydra fluctuans is a species common to eastern India. The plant has been used in Indian medicine in treatment of nervous ailments, skin diseases and as a laxative [1]. In addition to enhydrin (**1**) [2], fluctuadin (**2**), fluctuadin (**3**) [3] and other sesquiterpene lactones isolated earlier [4], we now report the isolation of two new chlorine containing melampolides (**4** and **5**) from the leaves of *E. fluctuans*.



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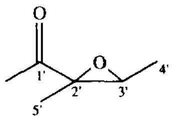

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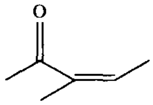

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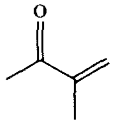

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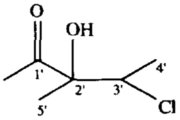

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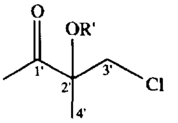
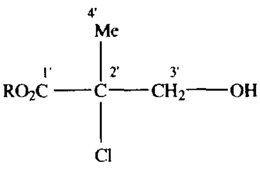
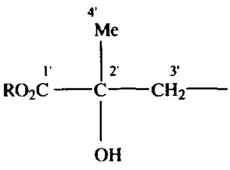


R'=H **5a** R'=OCOMe



$$RO_2\overset{1'}{C}-\overset{\overset{4'}{Me}}{\underset{Cl}{\overset{2'}{C}}}-\overset{3'}{CH_2}-OH$$

(I)



$$RO_2\overset{1'}{C}-\overset{\overset{4'}{Me}}{\underset{OH}{\overset{2'}{C}}}-\overset{3'}{CH_2}-Cl$$

(II)

RESULTS AND DISCUSSION

8-Deepoxyangeloyl-8-[3-chloro-2-hydroxy-2-methylbutyroyl] enhydrin (**4**) had distinctive IR bands in the carbonyl region at 1768 cm^{-1} from a γ-lactone, at 1738, 1711 from esters and 3450 cm^{-1} from a hydroxyl group. The mass spectrum showed the highest peak at *m/z* 441 accompanied by an isotopic peak at *m/z* 443, the ratio between the two being 3:1. Loss of HCl from *m/z* 441 could account for the signal at *m/z* 405. The compound also gave a positive response for chlorine in the Lassaignes test confirming the presence of chlorine. The base peak appeared at *m/z* 348 as in enhydrin and related compounds, due to the loss of the acyloxy group at C-8. This observation indicated that the compound has the same skeletal structure as enhydrin and that the chlorine atom must necessarily be present in the C-8 acyloxy moiety. The structure of this group was deduced from the ^1H NMR spectrum which showed a three proton singlet at δ 1.25, a three proton doublet at 1.47 and a one proton quartet at 4.07, in addition to other signals in common with enhydrin. Therefore, the structure of the acyloxy group could be deduced as 3-chloro-2-hydroxy-2-methyl butyroyl. This is compatible with the mass spectral fragment at *m/z* 135 which corresponds to the acylium ion arising from this moiety. The near identity of the rest of the ^1H NMR spectrum of **4** with that of enhydrin strongly suggests identical stereochemistry. This compound was prepared earlier by the action of hydrogen chloride on enhydrin [5]. A direct comparison of the natural sample of **4** with the one obtained from enhydrin showed the two to be identical in every respect (IR, NMR, MS). The absence of the molecular ion peak of **4**, but the appearance of a prominent fragment peak at *m/z* 441 was due to the loss of an acetoxyl group. Similarly, the spectrum of the acetate (**4a**) had the highest fragment peak *m/z* 483 due to the loss of a acetoxy group from the molecule.

8-Deepoxyangeloyl-8[2-hydroxy-3-chloro-isobutyroyl] enhydrin (**5**) had two distinctive carbonyl absorption bands, at 1752 cm^{-1} from a γ-lactone and 1740 cm^{-1} from an ester group. In addition it showed a sharp IR band at 3640 $cm^{-1}$from a hydroxyl group. The ^{1}H NMR spectral data of **5** also differed from those of **1** only in the signals of the ester moiety. The mass spectrum showed the highest peak at *m/z* 457 accompanied by an isotopic peak at *m/z* 455, the ratio between the two being 3:1, thus indicating the presence of a chlorine atom, which was confirmed by the Lassaignes test. The base peak at *m/z* 348 in the mass spectrum resulting from the loss of an acyloxy group at C-8 indicated no change in the main carbocyclic skeleton which was similar to that of **1**. The nature of the C-8 residue was deduced by analysis of the ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum of **5** showed two closely spaced one proton doublets with $J = 16.7$ Hz, due to the geminal protons at C-2′, in addition to a three proton singlet at δ1.36 and a one proton signal at 3.30, the latter being D_2O exchangeable. Further, ^{13}C NMR spectra revealed a carbonyl signal, a methyl resonance at δ23.3, a methylene resonance at 50.1 and a quaternary carbon signal at74.7. The data indicated the presence of a α-chloro-β-hydroxy isobutyrate (I) or a α-hydroxy-β-chloro isobutyrate (II) residue. The acetate (**5a**) showed only a small downfield shift of the methylene signals, but a large downfield shift of the quaternary carbon resonance in the ^{13}C NMR spectrum from δ74.7 in **5** to 78.5 in the acetate (**5a**), unambiguously indicating the presence of a α-hydroxy-β-chloro isobutyrate residue (II) in **5**. The absence of the molecular ion peak of **5** in the mass spectrum and the appearance of a prominent fragment peak at *m/z* 455 is due to the loss of a methoxyl group from the molecule. It was noticed that there was a considerable quantitative seasonal variation of the sesquiterpenes (other than enhydrin) in this plant. Compound **5** was present in larger quantities in the plant collected in the months of October and November, while **2** and **3** were not present in appreciable quantities. On the other hand, plants collected in June and July showed little or no **5**, while **2** and **3** were in appreciable quantities.

EXPERIMENTAL

Enhydra fluctuans was collected in Calcutta, West Bengal (India) in July 1990 and again in October 1992 by United Chemicals and Allied Products (Calcutta). The voucher specimen was deposited at the herbarium of SSSIHL (NO: EN 001).

The air-dried leaves (3 kg), collected in July, were exhaustively extracted with petrol (60–80°) for 18 hr. The combined extract (4 × 2.5 l) was concd to *ca* 150 ml. The green supernatant liquid was decanted off from the brown residue (6 g) which was dissolved in C_6H_6 and fractionated by CC (silica gel) with C_6H_6, mixts of C_6H_6–$CHCl_3$, $CHCl_3$ and mixts of $CHCl_3$–MeOH of increasing polarity. The C_6H_6–$CHCl_3$ (75:25) fr. yielded 20 mg of a mixt. of **2** and **3**. Purification by PTLC (CH_2Cl_2–C_6H_6–Et_2O, 10:9:2.5) yielded 2.3 mg of **2**. The $CHCl_3$–MeOH (98:2) fr. gave a mixt. which on purification by PTLC (C_6H_6–EtOAc, 70:30) gave 2.5 mg **4**.

The air-dried leaves (1.5 kg) collected in October, 1992, were extracted with petrol (60–80°) for 18 hr and worked-up as described above. The petrol insoluble material (10 g) was fractionated by CC (silica gel) with C_6H_6, mixts of C_6H_6–EtOAc of increasing polarity. The C_6H_6–EtOAc (75:25) fr. gave 1.5 g **1**. The C_6H_6–EtOAc (70:30, 65:35) frs gave **5** (225 mg).

8-*Deepoxyangeloyl*-8-[3-*chloro*-2-*hydroxy*-2-*methylbutyroyl*] *enhydrin* (**4**). Powder, mp 211–212°; UV λ_{max} nm (log ε): 220 (4.11). IR ν_{max} cm^{-1}: 3449 (OH), 1768 (lactone CO), 1734 (OAc), 1711, 1657 ($C=CH_2$), 1630 ($C=C–CO_2R$). ^{1}H NMR: Table 1. EIMS (probe) 70 eV *m/z* (rel. int.): 443 (2.8), 441 $[M - OAc]^+$ (8.3), 405$[441 - HCl]^+$ (2.4), 377 (15.2), 348 (100), 317 $[348 - OMe]^+$ (5.0), 229 (57.5), 135 $[RCO]^+$ (10.9), 109 (71.0), 107 $[135 - CO]^+$ (88.7), 71 (53.0), 55.3 (49.3); identical with the sample obtained by the action of methanolic HCl on **1**.

8-*Deepoxyangeloyl*-8-[2-*chloro*-3-*acetoxy*-2-*methyl butyroyl*] *enhydrin* (**4a**). Produced by acetylation of **4** with Ac_2O–pyridine. Powder. IR ν_{max} cm^{-1}: 1768 (lactone CO), 1737 (OAc), 1720 (OCOR), 1610, 865. EIMS (probe) 70 eV *m/z* (rel. int.); 487 $[M - OAc]^+$ (9.6), 364 $[M - RCO]^+$ (5.8), 348 $[M - RCO_2H]^+$ (100), 317 $[348 - OMe]^+$ (1.6), 289 $[317 - CO]^+$ (9.8), 229 (50.8), 177 $[RCO]^+$ (56.6), 149 (75.6), 55 (23).

8-*Deepoxyangeloyl*-8-[2-*hydroxy*-3-*chloro*-*isobutyroyl*] *enhydrin* (**5**). Needles, mp 209–210°; UV λ_{max} (log ε) nm: 224 (4.80). IR ν_{max} cm^{-1}: 3460 (OH), 1750 (lactone CO), 1740 (OAc), 1700, 1645. ^{1}H NMR: Table 1.

Table 1. ^{1}H NMR spectral data of **1**, **4**, **5**, and **5a** (at 90 MHz, ppm, TMS, $CDCl_3$)*

H	**1**	**4**	**5**	**5a**
1	7.16 *dd*	7.15 *dd*	7.14 *dd*	7.2 *dd*
5	2.69 *d*	2.66 *d*	2.65 *d*	2.64 *d*
6	4.28 *t*	4.30 *t*	4.26 *t*	4.22 *t*
7	3.03 *m*	2.98 *m*	3.08 *m*	3.03 *m*
8	6.69 *br d*	6.70 *br d*	6.70 *br d*	6.69 *br d*
9	5.87 *d*	5.85 *d*	5.84 *d*	5.73 *d*
13a	5.83 *d*	5.90 *d*	5.91 *d*	5.89 *d*
13b	6.31 *d*	6.36 *d*	6.37 *d*	6.37 *d*
14	1.72 *s*	1.70 *s*	1.70 *s*	1.67 *s*
16	3.84 *s*	3.83 *s*	3.83 *s*	3.81 *s*
18	2.07 *s*	2.03 *s*	2.03 *s*	2.08 *s*
3′a	3.02 *q*	4.07 *q*	3.64 *d*	3.68 *d*
3′b	—	—	3.47 *d*	4.00 *d*
4′	1.18 *d*	1.47 *d*	1.36 *s*	1.48 *s*
5′	1.46 *s*	1.25 *s*	—	—
OH			3.30 *s*	
OAc-2′				2.02 *s*

J (Hz); **1**; 1,2 = 5,6 = 10; 1,2 = 7; 8,9 = 8; 3′,4′ = 5.5; 13a,13b = 3.5; **4**; 1,2 = 5,6 = 10; 1,2 = 7; 8,9 = 8.5; 13a,13b = 3.3; 3′,4′ = 6.6; **5**; 5,6 = 9.6; 8,9 = 8.5; 13a,13b = 3.2; 3′a,3′b = 11.7; **5a**; 5,6 = 9.7; 8.9 = 8.5, 13a,13b = 3.2; 3′a, 3′b = 11.7.

*The assignments of the chemical shift positions were made with the aid of ^{1}H–^{1}H COSY spectra.

Table 2. ^{13}C NMR spectral data of **1**, **5**, and **5a** (at 22.56 MHz, ppm, $CDCl_3$)*

C	**1**	**5**	**5a**
1	149.0	149.3	149.2
2	35.3	35.0	35.2
3	24.7	24.4	24.5
4		59.0	59.3
5	62.5[a]	62.5	62.7
6	75.5	75.4	75.7
7	45.2	44.9	45.9
8	71.0[b]	72.1	71.4
9	70.2[b]	69.8	69.7
10	129.5[c]	129.6	130.1
11	133.0[c]	132.4	132.7
12	167.3	167.7	167.9
13	122.0	123.1	123.4
14	17.4	17.1	17.2
15	164.9	170.0	165.4
16	52.5	52.5	52.3
17	167.7	170.0	168.8
18	20.7	20.5	20.3
1′	169.6	172.3	170.7
2′	59.2	74.7	78.5
3′	59.6[a]	50.1	46.5
4′	19.0	23.3	21.0
5′	13.6		

* The assignments of ^{13}C NMR chemical shifts were made with the aid of DEPT spectra.

[a–c] Data may be exchangeable.

^{13}C NMR: Table 2. EIMS (probe) 70 eV *m/z* (rel. int.): 457 (5.5), 455 $[M - OMe]^+$ (1.6), 427 $[455 - CO]^+$ (12.5), 412 $[455 - MeCO]^+$ (0.23), 394 $[412 - CO]^+$ (1.15), 348 $[94 - HCl]^+$ (100), 334 $[455 - RCO]^+$ (0.69), 43 $[MeCO]^+$ (61.9).

8-*Deepoxyangeloyl*-8-[*2-acetoxy*-3-*chloro-isobutyroyl*] *enhydrin* (**5a**). Needles. UV λ_{max} (log ε) nm: 224 (4.06). IR ν_{max} cm^{-1}: 1750 (lactone CO), 1738 (OAc, CO_2R): ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. EIMS (probe) 70 eV *m/z*, (rel. int.): 528 $[M]^+$ (0.18), 497 $[M - OMe]^+$ (2.2), 469 $[497 - CO]^+$ (4.8), 454 $[497 - MeCO]^+$ (0.27), 348 $[M - RCO_2H]^+$ (100), 163 (38.1), 135 (41.9).

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