Tetrahedron. Vol. 28, pp. 2285 to 2298. Pergamon Press 1972. Printed in Great Britain

STUDIES ON INDIAN MEDICINAL PLANTS—XXVIII[1]

SESQUITERPENE LACTONES OF *ENHYDRA FLUCTUANS* LOUR. STRUCTURES OF ENHYDRIN, FLUCTUANIN AND FLUCTUADIN

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(*Received in the U.K.* 16 *November* 1971; *Accepted for publication* 5 *January* 1972)

Abstract—The structures of enhydrin (2) and the co-occurring sesquiterpene lactones, *viz.* fluctuanin (3) and fluctuadin (4) isolated from *Enhydra fluctuans* Lour have been established. The stereochemistry of enhydrin has been suggested from NMR data. Selective hydrolysis of the acetate and the glycidate group in 2 yielded the alcohols 11 and 16 indicating acyl migration during their formation. Acetylation of 11 led further to acetolysis of the glycidate ester function to afford the diacetate 17. The location of the two acyl groups in 2 (and also in 3 and 4) still remain to be settled.

ENHYDRA FLUCTUANS Lour (N.O. Compositae; tribe, Heliantheae), a small edible marshy shrub with a bitter taste, is the only species of this genus indigenous to India. It is claimed[2] to have uses as a laxative, anti-inflammatory, anti-bilious, demulcent and against skin and nervous affections. More recently, it has been shown to possess high folic acid activity.[3] Stigmasterol was the only constituent reported[4] from this plant prior to our chemical investigation. Since then, the isolation and characterization of a number of constituents have been published[5–9] from this and other laboratories.

We reported[6] the isolation of a new compound A, $C_{23}H_{28}O_9$, m.p. 201°, along with the major bitter principle, enhydrin, $C_{23}H_{28}O_{10}$, m.p. 184°. Compound A has now been shown to be a mixture of two difficultly separable components (Experimental) designated as fluctuanin, $C_{23}H_{28}O_9$, m.p. 161–163° and fluctuadin, $C_{22}H_{26}O_9$, m.p. 202–205°.

Structure 1 was originally assigned to enhydrin by Seshadri *et al.*[5,7] The spectral and other available data however enabled us to assign the germacranolide structure 2 to enhydrin.** Although its NMR data was very close to the 9,10-epoxy derivative 5a (m.p. 218°) of uvedalin (5) recently reported by Herz and Bhat,[10] the wide divergence between the m.ps of the two compounds and the results of hydrolysis experiments led us to believe that enhydrin would be isomeric with uvedalin epoxide, with the positions of the two acyl functions exchanged. Further attempts to establish

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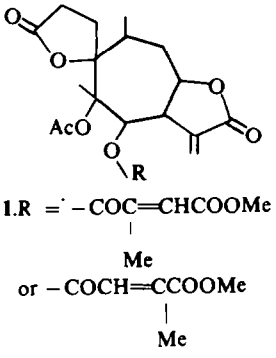
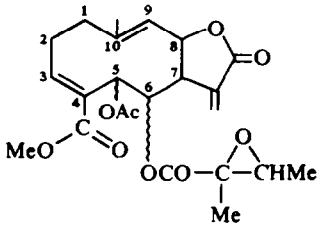
** Seshadri *et al.*[11] have revised the original structure (1) to 5a without conclusive evidence.

the positions of the acyl groups in enhydrin revealed an interesting case of acyl migration and acyl displacement which raised serious questions as to the validity of the conclusions drawn from hydrolytic experiments. It was thus thought desirable to carry out a direct comparison of enhydrin with uvedalin epoxide and their identity was indeed established† through the dihydroderivative **8**. Nevertheless the question of the location of the two acyl functions in enhydrin and for that matter in uvedalin was by no means settled. We therefore publish the full details of our work done so far on the structures of enhydrin (**2**), fluctuanin (**3**) and fluctuadin (**4**).





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5a 9,10-epoxide

The close relationship of the three compounds was revealed by their mass spectra (Table 1) showing a prominent common peak at *m/e* 348 for an ion of composition $C_{18}H_{20}O_7$ (Table 2) arising from loss of $C_5H_8O_3$, $C_5H_8O_2$ and $C_4H_6O_2$ from the

TABLE 1. SELECTED MASS SPECTRAL DATA OF **2**, **3** AND **4**

Compound	Common peaks Rel. intensity (%)					Other peaks *m/e* (Rel. intensity, %)		
	m/e 348	*m/e* 256	*m/e* 229	*m/e* 128	*m/e* 43	M^+	RCO^+	R^+
2	40	48	42	20	100	464(1)	absent	71(51)
3	12	3·5	4·5	7	59	448(2·5)	83(100)	55(41)
4	10	6	6	9	84	434(1)	69(100)	41(48)

TABLE 2. HIGH RESOLUTION MASS SPECTRAL DATA OF **3**

	m/e 448(M^+)	*m/e* 348	*m/e* 256	*m/e* 229	*m/e* 128	
Composition	$C_{23}H_{28}O_9$	$C_{18}H_{20}O_7$	$C_{15}H_{12}O_4$	$C_{14}H_{13}O_3$	$C_6H_8O_3$(95%)	$C_{10}H_8$(5%)
Measured mass	448·17351	348·12125	256·07366	229·08611	128·04743	128·06258
Calc. mass	448·17332	348·12089	256·07355	229·08644	128·04734	128·06260

† We are grateful to Prof. W. Herz for this and for informing us of the difficulty in arriving at an unequivocal conclusion on the identity of the original compounds since uvedalin epoxide is very difficult to distinguish from a stereoisomer isolated by them from another source. Joshi *et al.*[12] also recently reported the identity of enhydrin with uvedalin epoxide.

respective parent ions of **2**, **3** and **4**. That these expelled molecular units in the case of **3** and **4** involved C_4H_7COO— and C_3H_5COO— groups respectively were indicated by intense peaks for the corresponding acylium ions at *m/e* 83 and *m/e* 69 shifting to *m/e* 85 and *m/e* 71 respectively in their hydrogenated products. By analogy, the presence of a $(C_4H_7O)COO$— group was inferred in enhydrin (**2**). Though no peak for the five carbon acylium ion was observed in the mass spectrum of enhydrin, a peak at *m/e* 71 could be assigned to its decarbonylated species. The formulation of these units as 1-methyl-1,2-epoxybutyrate (glycidate group) in **2**, angelate in **3** and methacrylate in **4** could be arrived at from their NMR spectra (Table 3). The presence of the glycidate group in **2** was confirmed by preparation of the chlorohydrin **6** and the diol **7**.

An acetate group in enhydrin was indicated by IR absorption at 1740 cm^{-1} and an NMR signal at δ 2·07 (3H, s). The presence of an α-methylene-γ-lactone group could be inferred from absorptions at 1767 and 1667 cm^{-1} in the IR, while the NMR spectrum showed two one proton doublets (J = 3·5 Hz each) at δ 5·83 and 6·31, coupled (spin decoupling) to an allylic proton at δ 3·02. Catalytic hydrogenation of **2** over Pd/C gave two epimeric dihydro compounds (**8** and **9**) in which the NMR signals for the exomethylene group of **2** were replaced by that of a sec. Me group. The IR spectra of **8** and **9** showed absorptions for the lactone carbonyl at 1780 cm^{-1} and absence of the 1667 cm^{-1} band present in enhydrin.

The IR spectrum of **2** had a fourth carbonyl absorption at 1706 cm^{-1} and a C=C stretching band at 1635 cm^{-1} which was still present in the IR spectra of the dihydro compounds **8** and **9**. Moreover, the UV absorption of the dihydro compounds and a three proton singlet at δ 3·8 in the NMR spectrum of enhydrin showed the presence of a conjugated carbomethoxy group. Treatment of enhydrin with ethereal CH_2N_2 gave the major product **10** ($C_{25}H_{32}N_2O_{10}$; *m/e* 492, M^+-N_2). The IR (1780 cm^{-1}) and NMR (δ 4·75, m, —CH_2—N= in place of signals for =CH_2) spectra of the product indicated the formation of the expected spiropyrazoline involving the α-methylene group of the lactone. In addition, it exhibited a new vinyl Me signal at δ 1·96 in **10** in place of a one proton double doublet (J = 10·7 Hz) at δ 7·16 in **2**. This permitted the assignment of the last signal to the β-proton of the conjugated carbomethoxy group. Spin decoupling showed this proton to be coupled to two allylic protons resonating at δ 3·0 and 2·4. Thus, the partial structure A could be arrived at. The signal at δ 2·4 was part of a complex two proton multiplet. Spin decoupling indicated that the other proton of this complex and a proton at δ 1·23 constitute a second methylene group strongly coupled and hence adjacent to the one of partial structure A which could now be extended to B.

The functional groups so far discussed accounted for nine oxygen atoms and indicated enhydrin to be a sesquiterpene lactone having a single carbocyclic ring. Since there was no spectral evidence for OH group, the last oxygen atom in enhydrin could be present either as an ether or a ketone (no signal for aldehydic proton in the NMR). That it is in fact an epoxy group incorporated in partial structure C became evident from the following considerations. The NMR spectrum of enhydrin showed a

signal for CH_3—$\overset{|}{\underset{|}{C}}$—O group at δ 1·72 and a sharp one proton doublet (J = 10 Hz)

TABLE 3. NMR DATA[a] OF ENHYDRIN AND RELATED COMPOUNDS

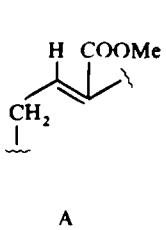
Compound	H-1	H-5	H-6	H-8	H-9	H-13	H-7	4-Me	COOMe	OCOMe	–OCOR			Others
											α-Me	β-Me	β-H	
2	7·16dd (10,7)	2·69d (10)	4·28t (10)	6·69dd (8,1)	5·87d (8)	6.31d(3·5) 5·83d(3·5)	3·02m	1·72	3·84	2·07	1·46	1·18d (5·5)	3·02q (5·5)	3·0m, H-2; 2·4m, H′-2 and H-3; 1·23m, H′-3.
2[b]	~7·32[c]	3·05d (9·5)	4·72t (9·5)	~7·23[c]	6·21d (9)	6·49d(3·5) 6·00d(3·2)	—	1·84	3·73	2·01	1·56	1·20d (5·5)	3·04q (5·5)	—
3	7·15dd (10,7)	2·68d (9·5)	4·29t (9·5)	6·74dd (8·5,1·5)	5·85d (8·5)	6·34d(3·5) 5.92d(3·0)	3·00m	1·69	3·82	1·99	1·78m	1·94d br(7)	6·08qq (7, 1·5)	3·0m, H-2; 2·4m, H′-2 and H-3; 1.22m, H′-3.
4	7·15dd (10, 7)	2·69d (9·5)	4·28t (9·5)	6·68dd (8·5, 1·5)	5·88d (8·5)	6·32d(3·5) 5·90d(3·5)	3·01m (9·5, 3·5, 3·5, 1·5)	1·72	3·82	1·98	1·89m	—	6·00m ($w_{\frac{1}{2}}$ = 3) 5·58 quin. (1·5)	3·0m, H-2; 2·4m, H′-2 and H-3; 1·23m, H′-3.
6	7·16dd (10, 7)	2·68d (9·5)	4·30t (9·5)	6·70dd (8, 1)	5·87d (8)	6·34d(3·5) 5.90d(3·5)	3·02m	1·70	3·83	2·03	1·30	1·48d (6·3)	4·09q (6·3)	3·20, OH
7	7·14dd (10, 7)	2·63d (9·5)	4·30t (9·5)	6·65dd (8, 1)	5·86d (8)	6.32d(3·5) 5·84d(3·5)	—	1·70	3·80	2·00	1·18	1·14d (6·5)	—	3·16, OH
8	7·16dd (10, 7)	2·58d (9·5)	4·13t (9·5)	6·30dd (8·5, 1)	5·88d (8·5)	1·31d(6·5)	—	1·69	3·82	2·02	1·50	1·29d (5·5)	3·06q (5·5)	—
9	7·13dd (10, 7)	2·58d (9·5)	4·49t (9·5)	6·31dd (8, 1)	5·83d (8)	1·17d (7·5)	—	1·69	3·83	2·02	1·49	1·29d (5·5)	3·06q (5·5)	—
10	—	2·76d (10)	4·54t (10)	5·86d (8)	5·64d (8)	4·75 m[d]	3·87d (10)	1·72	3·74	2·08	1·48	1·27d (5·5)	3·08q (5·5)	1·96, 1-Me
11	7·12dd (10, 7)	2·61d 9·5)	4·42t (9·5)	5·24dd br(8, 4)[e]	5·63d (8)	6·37d(3·5) 5·73d(3·5)	—	1·64	3·77	—	1·57	1·24d (5·5)	3.05q (5·5)	2·93d(4), OH
11[f]	7·27dd (10, 7)	2·94d (9·5)	4·92t (9·5)	5·72m[g]	6·13d (8·5)	6·43d(3·5) 5·79d(3·2)	—	1·80	3·73	—	1·54	1·32d (5·5)	3·02q (5·5)	—
12	7·12dd (10, 7)	2·56d (10)	4·31t (10)	4·96dd br(8, 4)[g]	5·62d (8)	3·68[i]	—	1·62	3·77	—	1·54	1·24d (5·5)	3·02q (5·5)	3·37, 13-OMe
13	6·90dd (10, 7)	2·61d (10)	4·32t (10)	4·50dd (8,1)[h]	4·24d (8)[h]	3·66[i]	~2·4m	1·53	3·75	—	—	—	—	3·00dt(12, 3, 3), H-11; 3.33, 13-OMe

14	7·14dd (10, 7)	2·63d (9·5)	4·13t (9·5)	6·23dd (8, 1)	5·82d (8)	3·73[i]	2·51m (12, 9·5, 1)	1·69	3·82	2·20 2·04	–	—	—	3·38, 13-OMe
15[f]	*c*	2·92d (10)	4·88t (10)	5·69d br(8)[g]	6·12d (8)	6·40d (3·5) 5·72d (3·5)	—	1·78	3·70	—	1·59	1·43d (6)	4·39q (6)	—
16	7·10dd (10, 7)	2·62d (10)	4·41t (10)	5·20m[e]	5·62d (8)	6·37d (3·5) 5·70d (3·5)	—	1·64	3·76	2·10	—	—	—	1·56, OH
17	7·12dd (10, 7)	2·65d (10)	4·27t (10)	6·61dd (8, 1)	5·79d (8)	6·32d (3·5) 5·82d (3·5)	2·96m	1·70	3·81	2·01 2·03	—	—	—	1·25m, H′-3
18	7·12dd (10, 7)	2.64d (10)	4·44t (10)	5·28m[e]	5·72d (8)	6·39d (3·5) 5·70d (3·5)	—	1·68	3·75	—	1·90br	1·95d br(7)	6·16q br(7)	1·52, OH
19	7·11dd (10, 7)	2·60d (10)	4·35t (10)	4·94dd (8, 4)	5·68d (8)	3·68[f]	2·4m	1·66	3·73	—	1·90br	1·98d br(7)	6·14q br(7)	2·72d(4), OH: 3·34, 13-OMe; 2·96dt (12.3.3). 11-H
20	7.16dd (10, 7)	2.57d (10)	4·13t (10)	6·22dd (8, 1)	5·86d (8)	1·32d (6·5)	—	1·68	3·82	2·00	1·13d (7)	0·90t (7)	—	—
21	7·15dd (10, 7)	2·56d (10)	4·12t (10)	6·20dd (8, 1)	5·86d (8)	1·32d (6·5)	—	1·69	3·81	2·00	1·14d(7) 1·13d(7)	–	—	—

[a] 100 MHz, recorded with a Varian HA-100 spectrometer in $CDCl_3$ solution with internal TMS standard. Chemical shifts are in δ units. Numbers in parentheses indicate coupling constants in Hz. [b] In Py-d_5 at 60 MHz. [c] Partially overlapped by β proton signals of solvent. [d] Seems to be a triplet (8 Hz) superimposed on a double doublet (9, 6 Hz). [e] Broadened doublet (8 Hz) after D_2O exchange. [f] In Py-d_5. [g] These signals have not been checked with D_2O exchange. [h] The pattern changes after D_2O exchange. The data recorded are for the D_2O exchanged spectrum. [i] Centre of two double doublets. Analysis as AB part of ABX spectra indicates $J_{AB} = 10$ Hz, $J_{AX} = J_{BX} = 3$ Hz and $\nu_A - \nu_B$ in the range 14 to 17 Hz.

at δ 2·69 (H_a) coupled* to another proton at δ 4·28 (H_b). The last signal was a triplet (J = 10 Hz) being coupled to the allylic proton of the α-methylene-γ-lactone at δ 3·02 (H_c). The last two signals in the NMR spectrum of enhydrin were a pair of AB doublets (J = 8 Hz) at δ 6·69 (H_d) and δ 5·87 (H_e) which must be assigned to a H—C—C—H unit. The low field proton H_d showed a small coupling (1 Hz) to

the allylic proton H_c. An alternative arrangement with H_d as the lactonic proton was considered unlikely in view of the small coupling (1 Hz) between H_c and H_d



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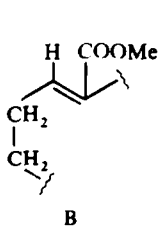

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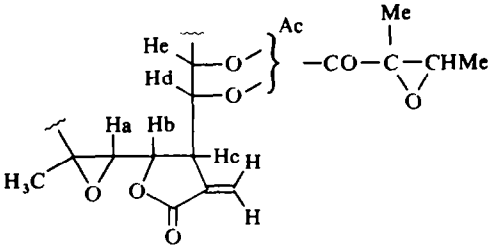


C

and was eliminated from chemical considerations involving hydrolysis experiments which are described in the sequel.

Now, the partial structures B and C accounted for all the carbon, hydrogen and oxygen atoms in enhydrin and, considering the multiplicity of the proton H_c they could be joined only in one way leading to structure **2** for enhydrin.

Enhydrin was found to be very sensitive to alkali and alkaline hydrolysis even under carefully controlled conditions yielded a number of products from which three (**11**, **12** and **13**) were definitely characterized. Analytical and spectral data showed that two (**11** and **12**) were partially hydrolysed products retaining the glycidate group while both acyl groups were hydrolysed in the formation of **13**.

The NMR spectra of **11** and **12** lacked the acetate Me signal. That only the C-8 centre was involved in the formation of **11** was apparent from replacement of the double doublet at δ 6·69 for the H-8 in **2** by a multiplet (broadened doublet after D_2O exchange) at δ 5·24. In **12** the H-8 signal was shifted upfield to δ 4·96 and the signals for the exomethylene group was replaced by those for a $—CH_2OCH_3$ group. In the NMR spectrum of **13** the signals for both the acetate and glycidate groups were absent. The signals for both H-8 and H-9 were shifted upfield to δ 4·50 and 4·24 respectively retaining their original multiplicity. Acetylation of **13** gave the diacetate **14**.

Treatment of enhydrin with $HClO_4$ aq. in THF, besides affording the diol **7** already mentioned, yielded its deacetylated product **15** which analysed for $C_{21}H_{28}O_{10}$

* All spin interactions except that involving H_d and H_e of the protons shown in the part structure C were checked by spin decoupling on **2**. Due to overlapping of the signals for H_c, the β proton of the glycidate moiety and one of the allylic protons of part structure B, the relationship of H_e and H_d could not be unambiguously established. However, in the spectrum of **4** the signal for H_e clearly showed a twelve line pattern arising from two allylic (J = 3·5 Hz each) and two vicinal (J = 9·5 and 1·5 Hz) couplings.

(M^+, *m/e* 440). Its IR spectrum showed absorptions for OH (3520 cm^{-1}), the lactone (1770, 1667 cm^{-1}), the conjugated carbomethoxy (1695, 1630 cm^{-1}) and an acyl (1730 cm^{-1}) function. The NMR spectrum of the compound (recorded in pyridine-d_5 due to insolubility in $CDCl_3$) showed the absence of the acetate function. The opening of the glycidate epoxy function was indicated by the downfield shift of the β-proton quartet to δ 4·39. The presence of the CH_3—CH(OH)—C(OH)—COO— group was

Me

further supported by the mass spectrum of **15** which showed loss of 44 mass units (CH_3CHO) from the molecular ion. The signals at δ 5·69 (bd, J = 8 Hz) and 6·12 (d, J = 8 Hz) were assigned respectively to $C_8\underline{H}$—OH and H-9 in comparison with the spectra of **2** and **11** in the same solvent.

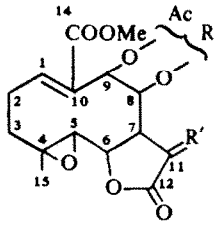
The structures of **11**, **12** and **15** indicated that the acetate and the glycidate group in enhydrin are located respectively at C-8 and C-9. Preferential elimination of the latter function was then expected to confirm this conclusion. Periodate oxidation of **7** followed by chromatography over silica gel yielded the amorphous alcohol **16**, the structure of which was ascertained from its spectral data. The IR spectrum showed absorptions for OH (3450 cm^{-1}), lactone (1761 cm^{-1}), acetate (1727 cm^{-1}) and carbomethoxy (1710 cm^{-1}) groups and the signals for the glycidate group were absent in the NMR spectrum. For the C-8 and C-9 protons it exhibited a sharp doublet at δ 5·62 for the CH_3COO—$C\underline{H}$— and a multiplet (bd on D_2O exchange) at δ 5·20 for the HO–$C\underline{H}$– protons. Since in enhydrin and its derivatives the doublet for the H-8 could be distinguished from H-9 by the small coupling to H-7, the OH group in **16** could only be placed at C-8. Indeed the NMR spectra of **11** and **16** were so similar that the OH group in both of them must be considered to be located in the same position, *i.e.* at C-8. Acetylation of **16** gave diacetate **17** the NMR spectrum of which showed all the features of enhydrin except that the signals for the glycidate group were replaced by an acetate.

The structure of **16** indicated that there must have been acyl migration during the formation of either **11**, **12** and **15** or of **16**. Reacetylation of **11** with Ac_2O in pyridine (expected to settle the question of acyl migration during the alkaline hydrolysis) resulted in an unexpected acyl displacement and the product was the diacetate **17**. Similar acetylation of **12** gave the diacetate **14**. Thus the exact locations of the acetate and the glycidate group in enhydrin (**2**) remain to be settled. Since similar acyl migration during selective removal of the glycidate group cannot be ruled out, the assigned positions of the two acyl functions in uvedalin[10] may not also be considered unambiguous.

It has already been shown that fluctuanin and fluctuadin contain angelate and methacrylate groups respectively in place of the glycidate group in **2**. Alkaline hydrolysis of fluctuanin (**3**) as in the case of **2** gave the deacetylated products **18** and **19**. The assigned structures follow from their analytical and spectral data. The major product of this hydrolysis was, however, the same amorphous diol **13** obtained from enhydrin.

Similar hydrolysis of fluctuadin (**4**) also gave **13** in good yield. The scale on which this reaction was carried out did not permit isolation of the partially hydrolysed products.

The common hydrolysis product **13** chemically correlated the three compounds



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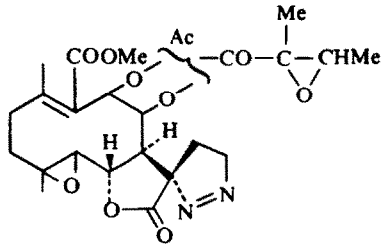


10

2. —CO—C(Me)(O)—CHMe — CH_2

3. —CO—C(Me)=CHMe — CH_2

4. —CO—C(Me)=CH_2 — CH_2

6. —CO—C(Me)(OH)—CHMe(Cl) — CH_2

7. —CO—C(Me)(OH)—CHMe(OH) — CH_2

8. —CO—C(Me)(O)—CHMe — H / Me

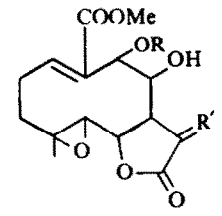
9. —CO—C(Me)(O)—CHMe — Me / H

14. Ac — H / CH_2OMe

17. Ac — CH_2

20. —CO—CH(Me)CH_2Me — H / Me

21. —CO—$CHMe_2$ — H / Me



R R′

11. —CO—C(Me)(O)—CHMe — CH_2

12. —CO—C(Me)(O)—CHMe — H / CH_2OMe

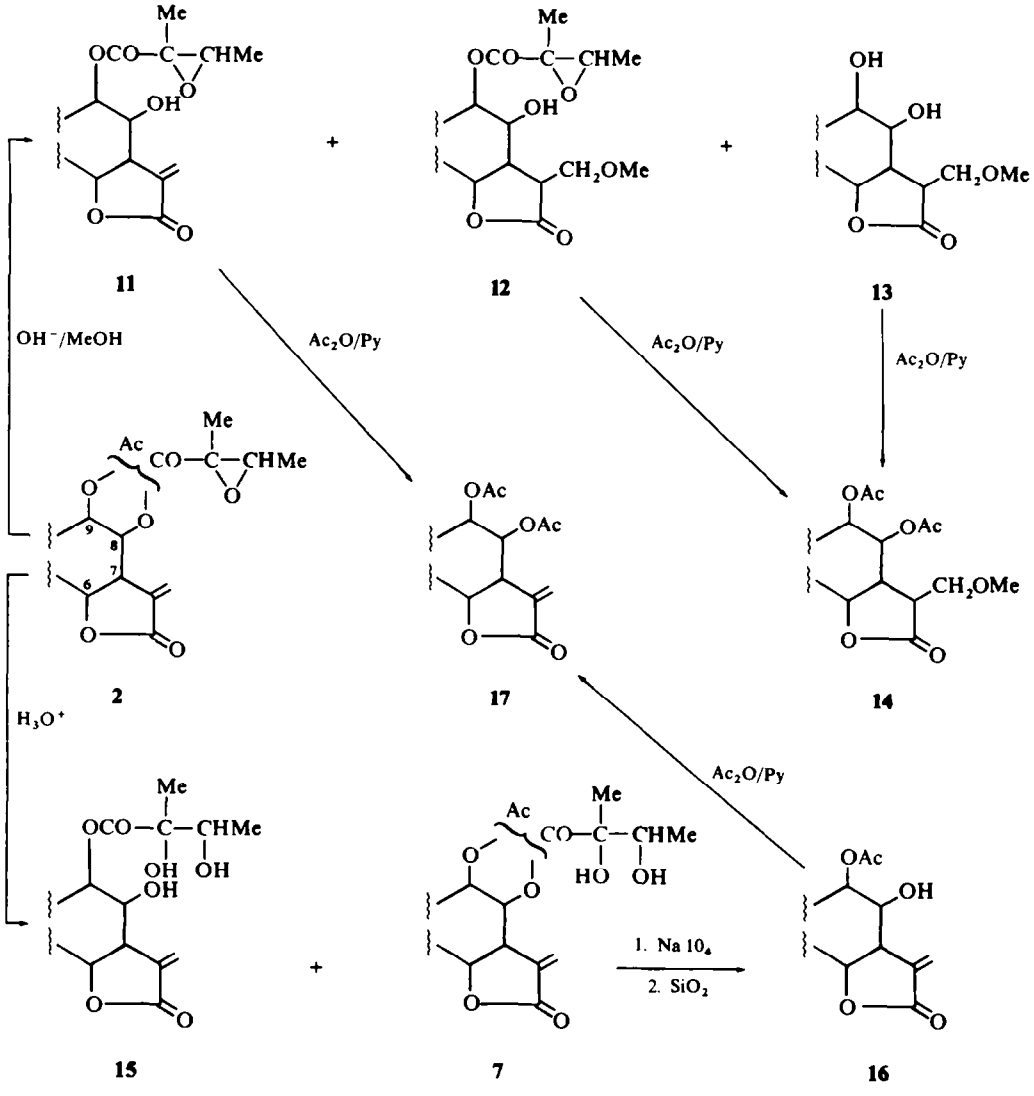
13. H — H / CH_2OMe

15. —CO—C(Me)(OH)—CHMe(OH) — CH_2

16. Ac — CH_2

18. —CO—C(Me)=CHMe — CH_2

19. —CO—C(Me)=CHMe — H / CH_2OMe

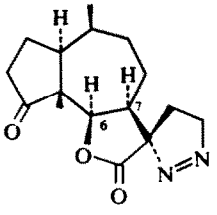

Me
OCO—C—CHMe
OH
O
CH2OMe
OH
11
12
13
OH⁻/MeOH
Ac2O/Py
Ac2O/Py
Ac2O/Py
Ac
CO—C—CHMe
OAc
2
17
14
H3O+
Ac2O/Py
HO
1. Na 10₄
2. SiO2
15
7
16

SCHEME

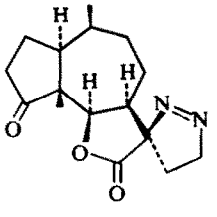
and established structure **3** for fluctuanin and **4** for fluctuadin. It does not necessarily follow that the positions of the acetate and the 4/5 carbon acyl groups are the same in all three compounds.

Due to difficulty in separation, a mixture of **3** and **4** was hydrogenated. From the two pairs of epimers formed, at least the major components could be separated in sufficient purity and characterized as the tetrahydrofluctuanin (**20**) and tetrahydrofluctuadin (**21**) derivatives.

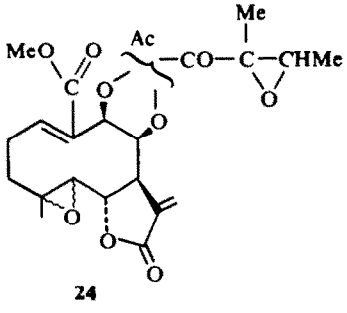
The NMR spectrum of the pyrazoline **10** indicated the lactone ring in enhydrin (**2**) to be *trans*. If it were *cis* the attack by CH_2N_2 would have been expected predominantly from the less hindered α-side (assuming the C_7—C_{11} bond to be β as in other sesquiterpene lactones of known stereochemistry). NMR spectrum of the pyrazoline would be similar to **22** (derived from damsin with known *cis* fused lactone) wherein H-6 has been shown[13] to be considerably deshielded (by 0·9 ppm) due to the anisotropy of the —N=N— bond while H-7 suffered a small downfield shift. No significant deshielding of H-6 was however observed in **10**, but the remarkable downfield shift (by 0·85 ppm) of H-7 could not be explained[13] by assuming its epimeric pyrazoline structure as in **23**. We, therefore, believe that the stereochemistry shown in **10** explains the NMR data of the pyrazoline derived from **2**. This conclusion is also in agreement with the molecular model (Drieding) of the compound. Herz and Bhat[10] suggested a *cis* lactone in uvedalin from the CD data of the compound itself and its major pyrazoline derivative assuming lactonisation at C-8. However their data is equally compatible[14, 15] with a *trans* lactone closed at C-6 as suggested here.



22



23



24

Another interesting feature in the NMR spectrum of **10** was the upfield shift of the H-8 signal* by 0·83 ppm. Such a shielding effect of the —N=N— bond has been observed[16] in simple pyrazolines and, in the present case, it indicates an α configuration for H-8. Consequently, the C-8 substituent in **2** could be assigned the β-configuration. Since the facile acyl migration and displacement in the partially hydrolysed products of **2** require eclipsed or near eclipsed orientation for the C-8 and C-9 substituents (a conclusion supported by the magnitude of the vicinal coupling constant between H-8 and H-9) the C-9 substituent in enhydrin may also necessarily be assigned the β configuration.

* In the spectrum of **10** the H-8 and H-9 signals appeared at δ 5·86 and 5·6 as sharp doublets. In the absence of the small vicinal coupling of H-8 to H-7, the assignment of the lower field signal to H-8 may not be considered unambiguous. However, even if it were otherwise the further upfield shift (1·04 ppm) would not affect the conclusions drawn in the sequal.

Thus, enhydrin could be assigned the stereochemistry shown in **24**. A comparison of the NMR data of costunolide,[17] and uvedalin[10] on the one hand and parthenolide[18] and enhydrin on the other indicates that in all probability the stereochemistry at C-4 and C-5 in these two series of compounds are identical.

The assignment of the configuration of the C_{11}-Me groups in **8** and **9** are based on the magnitude of $^3J_{11\text{-H, Me}}$ in their NMR spectra. It has been suggested[19] recently that in such epimeric lactones this coupling constant is larger in the epimer with *cis* related H-7 and H-11. Accordingly, C_{11}-Me group can be assigned the α configuration in **8** ($J_{H, Me}$ = 6·5 Hz) and the β in **9** ($J_{H, Me}$ = 7·5 Hz). The NMR spectra of **20** and **21** showed that their C-11 stereochemistry corresponded to that of **8** and not **9**. The —CH_2OMe group in the adducts **12**, **13**, **14** and **19** are also assigned the α configuration as addition of MeOH under alkaline condition generally leads to the thermodynamically more stable product. This was corroborated by the NMR spectrum of **13** in which the signal of H-11 appeared as a well resolved double triplet, with $J_{7, 11}$ = 12 Hz indicating *trans* relationship of the two protons.[20, 21]

EXPERIMENTAL

All m.p.s. were determined in a sulphuric acid bath in open capillaries and are uncorrected. Optical rotations in $CHCl_3$ and UV spectra in 95% EtOH solutions were measured in a Hilger-Watts M-511 Microptic photo-electric polarimeter and Uvispek (Model 007) spectrophotometer respectively. IR spectra were taken as nujol mulls (unless otherwise specified) with a Perkin-Elmer Infra-cord (Model 137) instrument. Mass spectra were recorded by Mr. R. G. Ross of Stanford University in an MS-9 spectrometer at 70 e.V. using the direct inlet system, the temperature of the ionisation chamber varying between 180° and 250°. High resolution mass measurements were done by the peak matching technique or by recording in real time with the ACME computer system of the Stanford University Medical School. Microanalyses were by Dr. R. D. Macdonald of Australian Microanalytical Service (CSIRO), Melbourne.

Petroleum ether, b.p. 60–80° and silica gel of M/s. Gouri Chemicals, Calcutta were used. Solvent systems employed for TLC were (A) C_6H_6:EtOAc (6:4) and (B) C_6H_6:EtOAc:HCOOH (60:40:2).

Isolation of enhydrin (**2**), *fluctuanin* (**3**) *and fluctuadin* (**4**). Dried and powdered whole plant (2 kg) of *Enhydra fluctuans* Lour. collected around Calcutta was extracted with petroleum ether in a Soxhlet apparatus for 30 hr, the extract concentrated to 150 ml and allowed to stand. The separated gummy mass (5·5 g) was chromatographed over silica gel (1·5 × 30 cm). The fractions eluted with C_6H_6 containing upto 10–20% $CHCl_3$ yielded 1·0 g of enhydrin (**2**), crystallized first from C_6H_6-pet. ether, then from EtOH as fine needles, m.p. 184°, $[\alpha]_D$ —54·7° (*c*, 1·17); ν_{max} 1767, 1751, 1740, 1706 (C=O), 1667. 1635 (C=C) cm^{-1}; λ_{max} 216 nm (ε, 13,600). (Found: C, 59·53; H, 6·32; 0, 34·10. $C_{23}H_{28}O_{10}$ requires: C, 59·47; H, 6·08; O, 34·45%).

The earlier fractions eluted with 60 to 80% C_6H_6 in pet. ether yielded a semi-crystalline mass (2·1 g) which on rechromatography over silica gel yielded 0·5 g of **2** and a crystalline mixture (0·6 g) of fluctuanin and fluctuadin. This mixture showed a single spot in TLC and separation was extremely difficult. Repeated chromatography over silica gel with IR monitoring eventually resolved it into its components. The faster moving was crystallized from EtOH to yield 0·1 g of fluctuanin (**3**) as fine needles, m.p. 161–163°, $[\alpha]_D$ −23·5° (*c*, 1·42); ν_{max} 1767, 1725, 1710sh (C=O), 1670, 1634 (C=C); λ_{max} 219 mm (ε 17,200), (Found: C, 61·28; H, 6·12; O, 32·74. $C_{23}H_{28}O_9$ requires: C, 61·59; H, 6·30; 0, 32·11%).

The slower component was crystallized from EtOH-pet. ether to yield 0·11 g of fluctuanin (**4**) as needles, m.p. 202–205°, $[\alpha]_D$—18·4° (*c*, 1·11); ν_{max} 1767, 1740sh, 1720 (C=O), 1667, 1634 (C=C) cm^{-1}; λ_{max} 216 nm (ε 17,150). (Found: *m/e* 434·1571. $C_{22}H_{26}O_9$ requires: *m/e*: 434·1577).

During chromatography of the mixture of **3** and **4**, evaporation of certain fractions left a spongy residue insoluble in common organic solvents. Its mass spectrum was however identical with that of **4**. A suspension of this material in DMSO was allowed to stand for a month when crystals of **4** having normal solubility characteristics were deposited. It was therefore considered to be a polymorphous form rather than a polymer of **4**.

Yields of the three compounds (especially **3** and **4**) varied from batch to batch of the plant material. During one extraction the appropriate fraction after separation of enhydrin and crystallization yielded a fraction composed predominantly (mass spec.) of **3**. This material was referred to as "Compound A" in an earlier communication.[6] In another extraction this particular fraction gave **4** uncontaminated by any **3**.

Chlorohydrin (**6**) *from* **2**. To a solution of 0·1 g of **2** in MeOH (5 ml) was added N HCl (5 ml). The mixture was stirred at room temp. for 12 hr. concentrated under reduced pressure, extracted with $CHCl_3$, washed, dried and distilled. The residue (95 mg) was chromatographed over silica gel. Elution with C_6H_6 followed by 90%C_6H_6 in $CHCl_3$ yielded **5** (58 mg), crystallized from EtOH-pet.ether in needles, m.p. 217°, $[\alpha]_D$—65·6° (*c*, 0·67); ν_{max} 3490 (OH), 1760, 1735, 1710 (C=O), 1653, 1626 (C=C) cm^{-1}; *m/e* 500, 502 (M^+). (Found: C, 55·25; H, 5·66; Cl, 7·00. $C_{23}H_{29}O_{10}Cl$ requires: C, 55·15; H, 5·83; Cl, 7·08%).

Dihydroenhydrins (**8**) *and* (**9**). Enhydrin (3·0 g) was hydrogenated over 10% Pd-C in EtOAc (100 ml) at room temp. and normal pressure for 6 hr. The product showing two spots in TLC was chromatographed over neutral alumina (activity I; 30 × 2·5 cm). The fractions eluted with C_6H_6 were crystallized from pet.ether-benzene to yield **8** (1·36 g) as fine needles, m.p. 221°, $[\alpha]_D$—80·0° (*c*, 0·78); ν_{max} 1780, 1750, 1740, 1710 (C=O) and 1640 (C=C) cm^{-1}; λ_{max} 217 nm (ε, 8,200); *m/e* 466 (M^+). (Found: C, 59·26; H, 6·30; $C_{23}H_{30}O_{10}$ requires: C, 59·22; H, 6·49%).

The fractions eluted with $CHCl_3$ on crystallization from pet. ether-C_6H_6 yielded **9** (1·45 g) as fine needles, m.p. 206–208°, $[\alpha]_D$—43·4° (*c*, 0·6); ν_{max} 1780, 1752, 1742, 1710 (C=O) and 1640 (C=C) cm^{-1}; λ_{max} 215 nm (ε, 7,300); *m/e* 466 (M^+). (Found: C, 58·85; H, 6·68. $C_{23}H_{30}O_{10}$ requires: C, 59·22; H, 6·49%).

Pyrazoline (**10**) *from* **2**. To a solution of **2** (0·1 g) in MeOH (10 ml) was added excess CH_2N_2 in Et_2O (10 ml) and the mixture allowed to stand at 5–10° for 24 hr. The separated crystals (60 mg) showing a single spot on TLC were recrystallized first from EtOH and then from C_6H_6—$CHCl_3$ to give **10** as fine needles, m.p. 172°, ν_{max} 1780, 1751, 1727, 1700 (C=O) and 1550 w (—N=N—) cm^{-1}; λ_{max} 227 and 317 nm (ε, 6,600 and 310); *m/e* 492 (M^+—N_2). (Found: C, 57·19, H, 6·22. $C_{25}H_{32}O_{10}N_2$ requires: C, 57·69; H, 6·20%).

Alkaline hydrolysis of **2**. A solution of 0·01 N KOH in 90% aqueous MeOH was added (drop per two min) under constant stirring to a solution of **2** (0·84 g) in MeOH (50 ml) at 0°. The addition of alkali continued until only a faint spot of starting material showed on TLC. The mixture was neutralized with Amberlite IR-120 (H^+ form) resin, filtered, evaporated and the residue chromatographed over silica gel. Elution with C_6H_6 and then 20% $CHCl_3$ in C_6H_6 gave a small amount of unconverted **2**. Elution with 50%$CHCl_3$ in C_6H_6 to $CHCl_3$ yielded 0·24 g (Fraction A) of a mixture of mainly two components. Further elution with $CHCl_3$ gave Fraction B (0·21 g) predominantly a single component.

Fraction A was rechromatographed on a short column of silica gel and the crystalline mixture subjected to PLC on silica gel with solvent system B. The faster moving band yielded **11** (8 mg) which crystallized from C_6H_6 as needles m.p. 245–247°, $[\alpha]_D$—63·3° (*c*, 0·6); ν_{max} 3410 (OH), 1754, 1740, 1715 (C=O), 1670 and 1634 (C=C) cm^{-1}; *m/e* 422 (M^+). (Found: C, 60·13; H, 5·99. $C_{21}H_{26}O_9$ requires: C, 59·71; H, 6·21%).

The slower band yielded **12** (25 mg) which crystallized from C_6H_6 in needles, m.p. 219–220°, $[\alpha]_D$—91·0° (*c*, 0·51); ν_{max} 3450 (OH), 1773, 1754, 1718 (C=O) and 1637 (C=C) cm^{-1}; *m/e* 454 (M^+). (Found: C, 58·12; H, 6·84. $C_{22}H_{30}O_{10}$ requires: C, 58·14; H, 6·66%).

Fraction B on similar PLC gave **13** (110 mg) as a foam which could not be crystallized but found homogeneous by TLC. ν_{max}^{dioxan} 3440 (OH) 1770, 1710 (C=O) and 1637 (C=C) cm^{-1}.

Diacetate **14**. (a) Acetylation of **13** (50 mg) with Ac_2O (0·5 ml) in pyridine (0·2 ml) at room temp. for 14 hr followed by usual work up and crystallization from C_6H_6-pet.ether yielded **14** (35 mg) as prisms, m.p. 196–197°, $[\alpha]_D$—100° (*c*, 0·69); ν_{max} 1790, 1750, 1733, 1721sh (C=O) and 1640 (C=C) cm^{-1}; λ_{max} 219 nm (ε, 5,670); *m/e* 440 (M^+). (Found: C, 57·61; H, 6·35. $C_{21}H_{28}O_{10}$ requires: C, 57·25; H, 6·41%).

(b) Compound **12** (25 mg) was acetylated identically and the solvent removed under vacuo. The residue on chromatography over silica gel and crystallization from pet.ether-C_6H_6 yielded **14** (14 mg) identical (m.p., m.m.p., IR) with the sample prepared from **13**.

Diol **7** *and triol* **15**. To a solution of **2** (1 g) in THF (20 ml) was added 3N $HClO_4$ (10 ml) and the mixture allowed to stand at room temp. The reaction was followed by TLC and proceeded until starting material almost disappeared. The products were extracted with $CHCl_3$, dried, distilled and the residue chromatographed over silica gel. Elution with 10–20% $CHCl_3$ in C_6H_6 yielded 50 mg of unconverted starting material. Elution with 70–90% $CHCl_3$ in C_6H_6 and then with $CHCl_3$ gave **7** (0·43 g) which crystallized from C_6H_6 as needles, m.p. 193°, $[\alpha]_D$—52.7° (*c*, 0·72); ν_{max} 3520, 3350 (OH), 1760, 1745, 1720 (C=O), 1667 and 1637 (C=C) cm^{-1}; *m/e* 482 (M^+). (Found: C, 57·55; H, 6·27. $C_{23}H_{30}O_{11}$ requires: C, 57·25; H, 6·27%).

The fractions eluted with 1-2% MeOH in $CHCl_3$ on crystallization from MeOH–$CHCl_3$ yielded **15** (0·13 g), m.p. 245–247°; ν_{max} 3520 (OH), 1770, 1730, 1695 (C=O), 1667sh and 1630 (C=C) cm^{-1}; *m/e* 440 (M^+). (Found: C, 57·10; H, 6·47. $C_{21}H_{28}O_{10}$ requires: C, 57·25; H, 6·41%).

Conversion of **7** *to* **16**. To a solution of **7** (0·36 g) in 70% aqueous THF (10 ml) was added 0·18 g of powdered $NaIO_4$, and the mixture stirred for 12 hr and extracted with ether, the extract was washed, dried and the solvent evaporated. The residue was chromatographed over silica gel. Elution with C_6H_6 and 10–20% $CHCl_3$ in C_6H_6 yielded 10 mg of a mixture showing a spot on TLC corresponding to the major product. Elution with 60–80% $CHCl_3$ in C_6H_6 followed by $CHCl_3$ gave a fraction containing a minor component (TLC) in the reaction mixture. Rechromatography of this fraction gave **16** as a colourless glass (0·16 g); $[\alpha]_D$—40·4° (*c*, 1·0); ν_{max} 3450 (OH), 1761, 1727, 1710 (C=O), 1637 (C=C) cm^{-1}; λ_{max} 217 nm (ε, 12,330); *m/e* 348 (M^+—H_2O).

Diacetate **17**. (a) Compound **11** (30 mg) was acetylated with Ac_2O (5 drops) in pyridine (3 drops) at room temp. Usual work up followed by chromatography over silica gel and crystallization from C_6H_6-pet.ether afforded **17** (18 mg) as fine needles, m.p. 210°, $[\alpha]_D$—38·6° (*c*, 0·58); ν_{max} 1770, 1748, 1730 (C=O), 1667 and 1640 (C=C) cm^{-1}; *m/e* 408 (M^+). (Found: C, 58·24; H, 5·81. $C_{20}H_{24}O_9$ requires: C, 58·81; H, 5·92%).

(b) Acetylation of **16** (25 mg) under the same conditions yielded 19 mg of **17** identical (m.p., IR) with the sample obtained from **11**.

Tetrahydrofluctuanin (**20**) *and tetrahydrofluctuadin* (**21**). A mixture of **3** and **4** (0·45 g) in EtOH (50 ml) was hydrogenated over 10% Pd-C for 12 hr at room temperature and atmospheric pressure. The product which showed two spots on TLC was resolved by chromatography over silica gel using 20 to 40% Et_2O in pet.ether as eluent The mass spectra however indicated both fractions to be mixtures of two components of mol. wt. 452 and 438. Repeated chromatography of the major fraction (0·25 g) yielded the two components **20** (25 mg) and **21** (58 mg), which were crystallized from pet.ether-Et_2O in needles. The NMR spectra showed that each still contained about 10% of the other. The samples had the following physical and analytical data: **20**: m.p. 161–163°; ν_{max} 1780, 1735, 1725, 1710 sh (C=O) and 1635 (C=C) cm^{-1}; λ_{max} 216 nm (ε, 8,200); *m/e* 452 (M^+) (Found: C, 60·45; H, 7·06. $C_{23}H_{32}O_9$ requires: C, 61·05; H, 7·13%). **21**: m.p. 141–143°, $[\alpha]_D$—92·5° (*c*, 0·54); ν_{max} 1780, 1740, 1725, 1710sh (C=O) and 1637 (C=C) cm^{-1}; λ_{max} 219 nm (ε, 7,120); *m/e* 438 (M^+). (Found: C, 60·50; H, 7·02. $C_{22}H_{30}O_9$ requires: C, 60·26; H, 6·90%).

The minor fraction of the original chromatography also showed molecular ion peaks at *m/e* 452 and 438 and from the NMR spectrum was a mixture of C-11 epimers of **20** and **21**. Attempts to resolve this mixture by chromatography were unsuccessful.

Alkaline hydrolysis of (**3**). Compound **3** (0·35 g) was hydrolysed in MeOH with 0·01 N methanolic KOH under conditions identical with those used in the case of **2**. The product on chromatography resolved into three fractions: Fraction A (47 mg), eluted with 30% $CHCl_3$ in C_6H_6; Fraction B (57 mg), eluted with 40 to 60% $CHCl_3$ in C_6H_6; Fraction C (151 mg), eluted with $CHCl_3$ and 2% MeOH in $CHCl_3$. All fractions were purified by PLC.

Fraction A afforded **18** (18 mg) crystallized from C_6H_6-pet. ether in needles, m.p. 179–180°, ν_{max} 3480 (OH), 1770, 1710, 1690 (C=O), 1650sh, 1635sh (C=C) cm^{-1}; *m/e* 406 (M^+). (Found: C, 61·84; H, 6·62. $C_{21}H_{26}O_8$ requires: C, 62·06; H, 6·45%).

Fraction B gave **19** (40 mg) crystallized from C_6H_6-pet. ether in needles, m.p. 191°; ν_{max} 3460 (OH), 1760, 1705, 1690 (C=O), 1640, 1635 (C=C) cm^{-1}; *m/e* 438 (M^+). (Found: C, 60·53; H, 6·66. $C_{22}H_{30}O_9$ requires: C, 60·26; H, 6·90%).

Fraction C yielded **13** (104 mg) as a colourless glass identical (IR) with the sample obtained from enhydrin. Acetylation of 30 mg of this sample with Ac_2O/pyridine yielded **14** (26 mg).

Alkaline hydrolysis of **4**. Hydrolysis of **4** (0·1 g) with 0·01 N methanolic KOH under the same conditions as above followed by chromatography and PLC of the product yielded **13** as major component (47 mg), the identity of which was further confirmed by converting to the diacetate (**14**). The minor products from this hydrolysis could not be investigated due to paucity of material.

Acknowledgment—A.M.D. is grateful to the National Institute of Health, U.S. Public Health Service (Grant No. AM-04257) and to the National Aeronautics and Space Administration (Grant No. NGR 05-020-004) for financial support. We also thank the Director, Indian Institute of Experimental Medicine and the Council of Scientific and Industrial Research, New Delhi for a Senior Research Fellowship to E.A.

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