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Structure of Enhydrin, a Germacranolide from *Enhydra fluctuans* Lour.*

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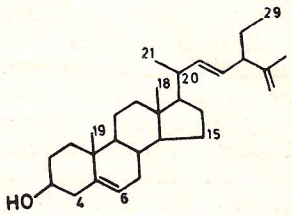
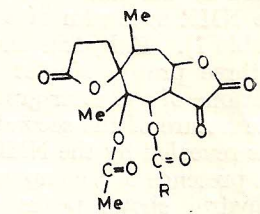
Based on spectral and chemical evidence, enhydrin ($C_{23}H_{28}O_{10}$, m.p. 185-6°, M^+ 464), a germacranolide isolated from the stem and leaves of *Enhydra fluctuans* Lour., has been shown to have the structure (IV). The lactone grouping together with three ester functions account for nine oxygen atoms. The tenth oxygen atom is involved in epoxide formation. The placement of the γ-lactone ring with respect to the epoxide and the two acyl groups has been decided on the spin-decoupling experiments. Selective hydrolysis of enhydrin and dihydroenhydrin confirms the presence of the acetoxyl at C-5 and α-methyl-α,β-expoxybutyryl residue at C-6. Enhydrin resembles in many respects (TLC, IR and NMR) with uvedalin epoxide isolated earlier from *Polymnia uvedalia* Linn., but differs in melting point. Dihydroenhydrin is however, different from dihydrouvedalin epoxide. This is expected as these are epimeric. (24*S*)-Ethyl-cholesta-5,22,25-triene-3-β-ol (I) has also been isolated from this plant.

DURING the course of our work on the chemical investigation of pharmacologically important Indian plants[1], we examined the stem and leaves of *Enhydra fluctuans* Lour. (Compositae), a small shrub growing in East Bengal and Assam[2]. Previous investigations on the chemical constituents of this plant have established the presence of stigmasterol[3], myricyl alcohol[4], kauran-16-ol[4], kaur-16-en-19-oic acid[4,5], 16-α-hydroxykauran-19-oic acid[6,7], 15-α-hydroxy-kaur-16-en-19-oic acid and its isovalerate and angelate[8] esters.

We wish to report here the results of our investigations on the stem and leaves of *E. fluctuans*. The hexane extract after concentration to small bulk gave a semi-crystalline residue. The filtrate on chromatographic separation on alumina gave a crystalline compound, m.p. 148°, which showed blue-green colour with Liebermann-Burchard reagent. It analysed for the formula $C_{29}H_{46}O$ (M^+ 410) and showed in the IR spectrum peaks at 890, 1650 ($\rangle C{=}CH_2$), 960 (*trans*-disubstituted double bond) and 800 cm^{-1} (trisubstituted double bond). It formed a monoacetate, m.p. 143° and a monobenzoate, m.p. 138°. The NMR spectrum of the sterol exhibited signals at δ 0·69 (*s*, 3H, C_{18} $\geqslant CH_3$), 0·81 (*t*, $J = 7$ Hz, 3H, $C_{29}{-}CH_2{-}CH_3$), 1·0 (*s*, 3H, C_{19} $\geqslant CH_3$), 1·02 (*d*, $J = 6$ Hz, 3H, $C_{21}{-}CH{-}CH_3$), 1·56 (*s*, OH, exchanges with D_2O), 1·65 (*s*, 3H, C_{27} $\geqslant CH_3$), 3·5 (*m*, 1H, $C_3{-}H$), 4·68 (*s*-broad, 2H, $\rangle C{=}CH_2$), 5·2 (*m*, 2H, $-CH{=}CH-$), and 5·3 (*m*, 1H, $C_6{-}H$). A survey of the literature showed it to be a new sterol probably containing an exocyclic methylene at C-25 and two double bonds at $C_5{-}C_6$ and $C_{22}{-}C_{23}$. When the present work was in progress, Rees *et al.* reported the isolation of two new sterols from *Clerodendron campbellii*, one of which was (24*S*)-ethyl-cholesta-5,22,25-trien-3-β-ol (I)[9]. A direct comparison of the m.m.p., TLC, IR and mass spectra, co-chromatography on two gas-liquid chromatography systems (QF-1 and SE-30)



(I)



(II) R = -C=CH-COOMe
Me

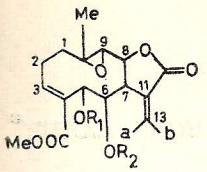
(III) R = -CH=C-COOMe
Me

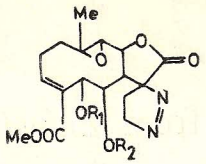
and NMR spectra of our compound with (**I**) showed their identity. Sucrow *et al.* have recently reported the synthesis of (**I**)[10].

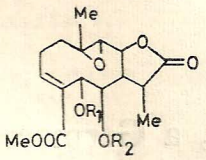
Chromatographic separation of the hexane extracted solids gave a colourless crystalline compound, m.p. 183-4° (shrinking at 122°). Seshadri and coworkers have reported the isolation of a sesquiterpene lactone enhydrin, m.p. 185-6° from the petroleum ether extract of *E. fluctuans*[11]. Mostly based on the NMR and mass spectral data they constituted enhydrin as (II or III)[12]. The compound isolated by us appeared to be identical with enhydrin as indicated by its molecular formula $C_{23}H_{28}O_{10}$ (M^+ 464), some of the mass-spectral fragments and the NMR signals reported for the compound[10,11].

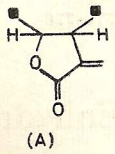
We wish to report our findings that enhydrin is not the proposed sesquiterpene spirolactone (II or III), but is a germacranolide having the structure (IV). Enhydrin showed an UV maximum at 211 nm (log ε, 4·24) and IR maxima at 1778 (conjugated γ-lactone), 1758, 1742 and 1710 cm^{-1} (ester or lactone). The existence of three ester functions and an α,β-unsaturated γ-lactone group in enhydrin could be inferred from the IR spectrum. This is also supported by the consumption of 3·8 equivalents of alkali on hydrolysis. The presence of an exocyclic methylene group conjugated with the γ-lactone (A) was shown by formation of the pyrazoline (V).

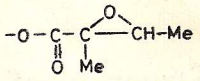
*Contribution No. 267 from CIBA Research Centre.



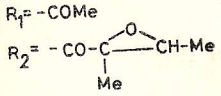




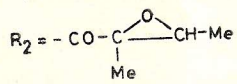




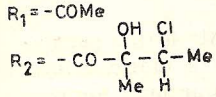
(IV) R_1 = -COMe



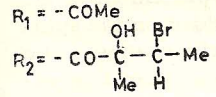
(V) R_1 = -COMe



(VII) R_1 = -COMe



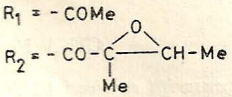
(VIII) R_1 = -COMe



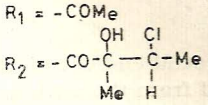
This was also confirmed by hydrogenation of the exocyclic methylene double bond to give dihydroenhydrin (VI) $C_{23}H_{30}O_{10}$ (M^+ 466), m.p. 196°, $[\alpha]_D$ −38°. The gross germacranolide structure was indicated by the functional groups as interpreted from the NMR spectrum of enhydrin and its derivatives (Table 1). Enhydrin and all its derivatives exhibit a three proton singlet around δ 2·0 suggesting that one of the ester groupings is due to an acetate. The nature of the second acyl grouping of enhydrin was revealed by the NMR spectrum which indicated the presence of a component (B). Mass spectrum of enhydrin shows peaks at *m/e* 405 and *m/e* 349 (base peak) due to the loss of M—59 and M—115 respectively, confirming the presence of acetyl and α-methyl-α,β-epoxybutyryl groups. By the action of hydrochloric acid in methanol, enhydrin gave a chlorohydrin (VII) by opening of the epoxide ring of the side chain (B). The structure (VII) was supported by its mass spectrum which shows peaks at *m/e* 441 and *m/e* 249 due to loss of 59 ($C_2H_3O_2$) and 151 ($C_5H_8O_3Cl$) units from the molecular ion (M^+ 500). The NMR spectrum of (VII) shows a downfield shift of the $C_{3'}$-proton from δ 3·0 to 4·12 and a similar shift of the $C_{3'}$-methyl doublet from δ 1·18 to 1·5. Similarly, treatment of enhydrin with hydrobromic acid resulted in opening of the epoxide of the side chain giving the bromohydrin (VIII).

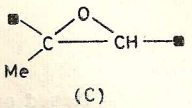
Dihydroenhydrin (VI) on treatment with hydrochloric acid gave the corresponding hydrogen chloride addition compound (IX). The third ester component is an α,β-unsaturated carbomethoxy group (a vinyl proton at δ 7·2 and a three proton singlet at δ 3·83). The lactone grouping together with the three ester functions account for nine oxygen atoms. Since enhydrin does not show the presence of a hydroxyl or carbonyl group, the tenth oxygen should be an ether and in all probability, an epoxide. The three-proton singlet at δ 1·7 is due to a methyl group carrying an epoxide as in (C). From the above mentioned facts, it follows that enhydrin should be a germacranolide. A partial structure (X) could then be deduced for enhydrin. The placement of the γ-lactone ring with respect to the epoxide and the two acyl groups in the ten-membered ring was shown by spin-decoupling experiments (Fig. 1). Irradiation of the frequency of H-9 at δ 2·7 (proton on the epoxide) converted only the triplet of H-8

(VI) R_1 = -COMe



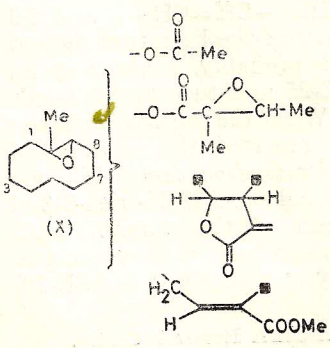
(IX) R_1 = -COMe



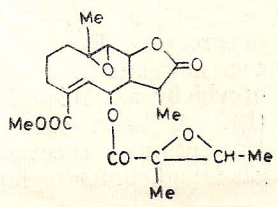


at δ 4·3 to a doublet. The lactone oxygen should, therefore, be placed next to the C-9 carbon carrying the epoxy group and the γ-lactone is thus attached at C_7-C_8. Irradiation of H-7 multiplet at δ 3·0 also collapsed the triplet of H-8 to a doublet and converted the doublet of doublets at δ 6·7 due to H-6 proton to a sharp doublet. This irradiation experiment (solvent, DMSO-*d*) also brought about a change in the exocyclic methylene protons of the lactone ring. The long range allylic coupling (3 Hz) of the *a* and *b* protons of the methylene group with C-7 proton disappeared and gave rise to two sharp singlets. Since one of the C-2 protons has signals at δ 3·0, the irradiation of this signal also affected partially the vinyl proton H-3 appearing at δ 7·2. Irradiation of the proton at δ 7·2 due to H-3 affected signals in the methylene region at δ 3·0 and 2·4. Also, irradiation of the C-3′ secondary methyl signal at δ 1·18 brought about a change in the signal at δ 3·0.

All the above mentioned data could be accommodated only in structure (IV) except for the ambiguity of placement of the acyl groups at C-5 and C-6 positions. Attempts were, therefore, made to selectively hydrolyse enhydrin (IV) and dihydroenhydrin (VI). However, a complex mixture of compounds was obtained. Reaction of enhydrin with sodium borohydride in methanol at 0° for 1 hr afforded, on chromatographic separation, a crystalline compound $C_{21}H_{28}O_8$ (M^+ 408), m.p. 213°. This has been assigned the structure (XI) on the basis of the UV spectrum which shows λ_{max} 220 nm (log ε 3·87) and IR (nujol) bands at 1780, 1740 and 1700 cm^{-1}. The NMR spectrum indicated that the exocyclic methylene group and the C_3-C_4 double bond of the allylic ester were reduced. Loss of the acetyl group could also be inferred by the absence of the signal at δ 2·0. In the methyl region two sharp singlets at δ 1·51 and 1·59 integrating for six protons showed two tertiary methyl groups



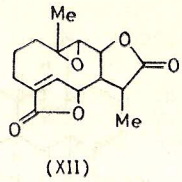
(X)



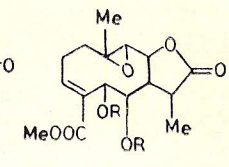
(XI)

TABLE 1 — NMR SPECTRA OF ENHYDRIN AND ITS DERIVATIVES

Compound	H-3	H-5	H-6	H-7	H-8	H-9	H-13	H-3′	$COOCH_3$	$COCH_3$	CH_3		
											C_{10}	C_2'	C_3'
IV	7·2 (q; 7, 10·2)	5·89 (d; 9)	6·7 (d, d; 9, 1)	3·0(m)	4·3 (t; 10)	2·7 (d; 10)	(a) 5·85 (d; 3) (b) 6·3 (d; 3·5)	3·0 (q; 5)	3·82 (s)	2·06 (s)	1·7 (s)	1·45 (s)	1·18 (d; 5)
V	7·2 (q; 7, 10·2)	5·83 (s)	5·83 (s)		4·75 (t; 9)	2·8 (d; 10)		3·1 (q; 5)	3·8 (s)	1·99 (s)	1·72 (s)	1·5 (s)	1·3 (d; 5)
VI	7·2 (q; 7, 10)	5·85 (d; 9)	6·32 (d; 9)	2·8(m)	4·5 (t; 10)	2·6 (d, 9·5)	1·2 (3H; d; 6·5)	3·1 (q; 5)	3·82 (s)	2·0 (s)	1·7 (s)	1·5 (s)	1·3 (d; 5)
VII	7·2 (q; 7, 10)	5·9 (d; 8·5)	6·7 (d; 8·5)	3·05(m)	4·3 (t; 10)	2·68 (d; 10)	(a) 5·95 (d; 3) (b) 6·35 (d; 3·5)	4·12 (q; 6)	3·82 (s)	2·05 (s)	1·72 (s)	1·3 (s)	1·5 (d; 6)
VIII	7·2 (q; 7, 10)	5·9 (d; 9)	6·7 (d; 8·5)	3·05(m)	4·35 (t; 10)	2·7 (d; 10)	(a) 5·95 (d; 3) (b) 6·35 (d; 3·5) 1·32	4·2 (q; 6)	3·82 (s)	2·05 (s)	1·75 (s)	1·32 (s)	1·69 (d; 6)
XI		5·9(m)	6·4(m)		4·15 (t; 10) 4·2(m)	2·6 (d; 10)	(3H; d; 6) 1·25 (3H; d; 6·5)	3·1 (q; 5)	3·8 (s) 3·75 (s)		1·59 (s) 1·55 (s)	1·53 (s)	1·32 (d; 6)
XIII	7·0(m)												
XIV	7·2 (q; 7, 10)	5·81 (d; 8)	6·2 (d, d; 9, 1)		4·15 (t; 10)	2·6 (d; 10)	1·3 (3H; d; 6)		3·82 (s)	2·08 (s) 2·0 (s)	1·67 (s)		



(XII)



(XIII) R = H

(XIV) R = -COMe

and the broad doublet of 5·5 Hz centred at δ 1·35 integrating for six protons, should be attributed to two secondary methyls. The carbomethoxyl group showed a sharp signal at δ 3·8. The broad doublets appearing at δ 5·9 and 6·4 could be attributed to the C-5, C-6 protons. The loss of the acetoxyl group could be explained by initial hydrolysis and subsequent dehydration to give the α,β-unsaturated ester (XI). The formation of (XI) proves the location of the acetoxyl group at C-5 and the α-methyl-α,β-epoxybutyryl group at C-6. By the reaction of enhydrin with sodium borohydride in methanol under reflux, a dilactone $C_{15}H_{18}O_5$ (XII) m.p. 260° was formed. The structure of this is based on the basis of its elemental analysis, UV (λ_{max} 217 nm), and IR (1780, 1750 cm^{-1}) spectra. Dihydroenhydrin (VI) on hydrolysis with sodium methoxide gave a crystalline diol (XIII) m.p. 210°, the structure of which is in agreement with the spectral data. This gave on acetylation, a diacetate $C_{20}H_{26}O_9$ (XIV), m.p. 255-7°.

When this work was in progress, Herz *et al.*[13] reported the structure elucidation of uvedalin (XV) isolated from the leaves of *Polymnia uvedalia* (L.) Linn. Dihydrouvedalin (XVI) was obtained by sodium borohydride reduction of (XV). Both the compounds were converted to the corresponding epoxides, m.p. 218-20° and m.p. 213-15°, and formulated by the gross structures (IV) and (VI) respectively. Although the melting point of enhydrin is different from that of uvedalin epoxide, we have compared their TLC, IR (KBr) and NMR spectra and found them to be identical in all respects*. Comparison of dihydroenhydrin (VI) with dihydrouvedalin epoxide prepared by sodium borohydride reduction of uvedalin and subsequent epoxidation showed them to be different. This could be expected as these are epimeric*. In a recent communication Seshadri *et al.* have withdrawn their earlier structure (II) or (III) and suggested (IV) as a structure for enhydrin[14].

Enhydrin is a rare example of such a highly oxygenated sesquiterpene lactone[15]. As regards the stereochemistry of enhydrin, it may not be simple to arrive at conclusions on the basis of physical methods alone and we have, therefore, initiated X-ray crystallographic work on the compound (VIII). Herz *et al.* have assumed a β-configuration for the C-11 side chain of uvedalin[13]. On the basis of a strongly negative Cotton effect exhibited by (XV) and the empirical rule relating to the sign of the lactone Cotton effect[16], a *cis* fusion of the γ-lactone ring has been proposed. However, the magnitude of the allylic interactions of the C-13 protons in

*Professor Herz has informed us on 22 February 1971 that uvedalin epoxide m.p. 218-20° reported in their paper was reached only once, and generally the m.p. is in the region 180-84°.

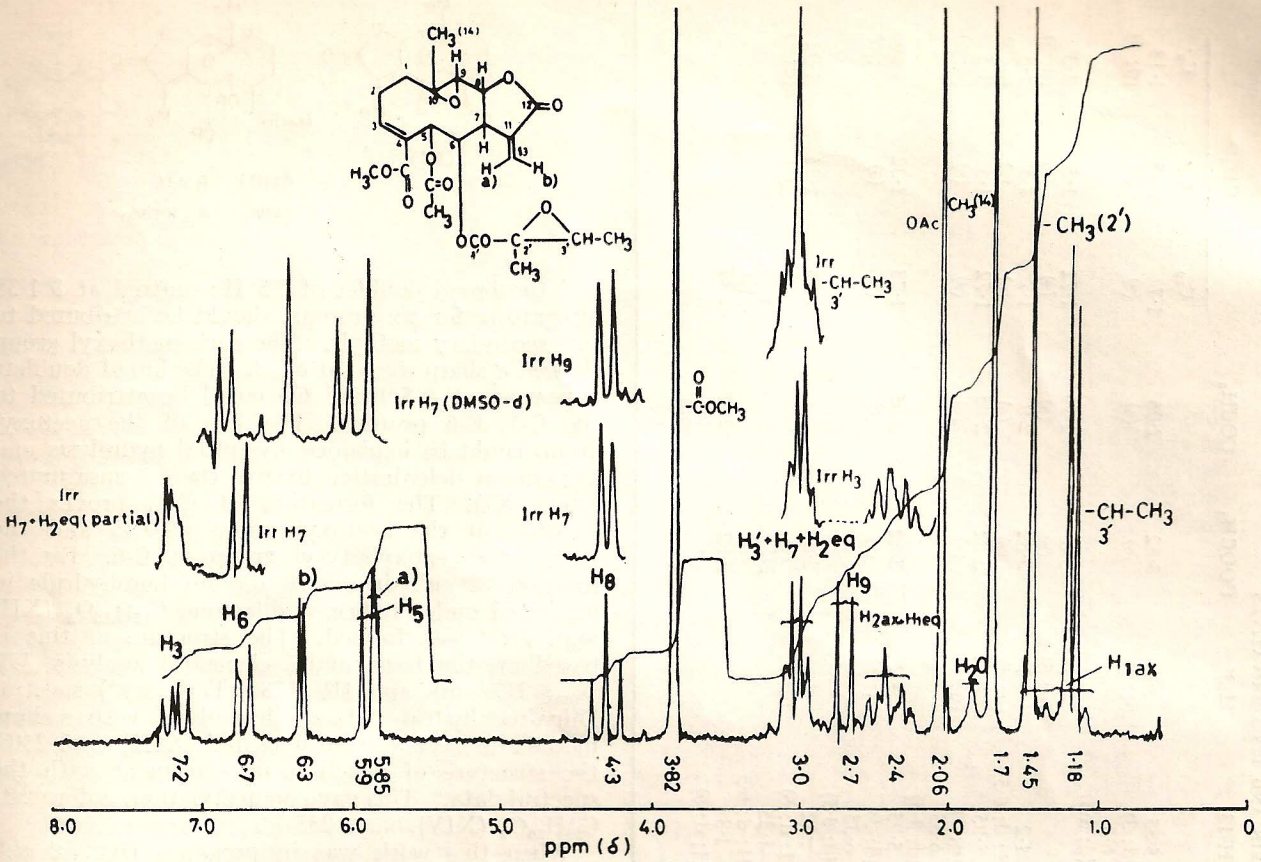
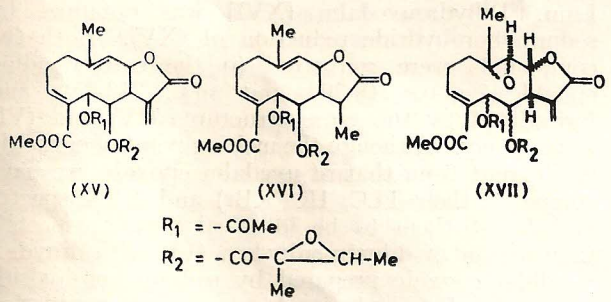


Fig. 1 — 100 MHz NMR spectrum of enhydrin (IV) in $CDCl_3$



enhydrin (IV) and its derivatives (VII), (VIII) and also uvedalin (XV)[13], ($J_{13,7} \geq 3$ Hz) is indicative of the presence of a *trans* fused γ-lactone[17,18].

The magnitude of the H_7-H_8 interaction ($J_{7,8}$ = 10 Hz) in enhydrin and its derivatives is suggestive of quasi-diaxial configuration for H-7 and H-8. Also, the coupling between the H_8-H_9 ($J_{8,9}$ = 10 Hz) shows that the H-7 is α-oriented. The NMR data can be interpreted by structure (XVII) or its mirror image, assuming a β-configuration for the C-11 side chain.

Experimental Procedure

Melting points are uncorrected. UV spectra were determined in ethanol using a Beckmann model DK-2A spectrophotometer. IR spectra were determined on a Perkin-Elmer model 421 or 337 spectrophotometer and optical rotations were taken in $CHCl_3$ solutions at 23°. NMR spectra were run on a Varian A-60 or HA-100-D spectrometer with TMS as an internal standard. The chemical shifts are expressed in δ values and figures in brackets are line separations in Hertz. Mass spectra were recorded at 70 eV using the direct inlet system on an Atlas CH-7 mass spectrometer. Merck silica gel was used for TLC and for detection the thin-layer chromatoplates were sprayed with a 1% solution of vanillin in aq. H_2SO_4 (1:1) and heated to 110° for 5 min.

Isolation of enhydrin (IV) — Dried and milled leaves and stem (19 kg) of *E. fluctuans* obtained from United Chemical and Allied Products, Calcutta were extracted with hexane (6×60 litres) by cold percolation. The extracts were concentrated under vacuum to 2 litres and left overnight at room temperature. The gummy solid was filtered, washed with hexane and dissolved in methanol. The MeOH soluble fraction was evaporated to dryness and triturated with ether when a semicrystalline solid separated (44 g). This was dissolved in $CHCl_3$ and chromatographed over neutral alumina (440 g) packed in $CHCl_3$. Elution with $CHCl_3$ gave enhydrin (27 g) which was recrystallized from $CHCl_3$-hexane. Enhydrin sublimes at 130°/10^{-2} mm, R_f 0·46 (silica gel; $CHCl_3$-2% MeOH), m.p. 183-4° (with shrinking at 122°); $[\alpha]_D$ −55·6° (*c*, 2·22) (Found: C, 59·4; H, 6·1. $C_{23}H_{28}O_{10}$ requires C, 59·5; H, 6·1%); M^+ 464.

Isolation of (24*s*)-*ethyl-cholesta*-5,22,25-*trien*-3-β-*ol* (I) — The hexane filtrate after removal of the gummy solids containing enhydrin, was chromatographed on neutral alumina (1 kg). The column was eluted with hexane and 100 ml fractions were collected and the development followed by TLC. The first 27 fractions gave a waxy material. Elution with C_6H_6 (fractions 28-35) gave a compound which

rystallized from MeOH as colourless needles (I; 2 g), p. 148°; $[\alpha]_D$ −38° (*c*, 2·02); IR (nujol) 3440, 00, 1650, 1255, 1240, 1190, 1170, 1130, 1100, 60, 1050, 1020, 1005, 985, 960, 890, 835 and 0 cm^{-1} (Found: C, 84·6; H, 11·5. $C_{29}H_{46}O$ requires 84·8; H, 11·3%); mass spectrum: *m/e* 410 (M^+), 5, 381, 363, 300, 272, 271, 255, 237, 213, 159, 5, 137, 109. R_f, 0·5 (silica gel; $CHCl_3$-2% MeOH); C retention time 9·2 min [Varian aerograph del 712, column 4 m×8 mm consisting of SE-30 5%) on chromosorb W (60-80 mesh) operated at with a flow rate of 125 ml/min N_2.] It formed cetate (Ac_2O in Py), m.p. 143°; $[\alpha]_D$ −36·5° 1-18) (Found: C, 81·7; H, 10·9. $C_{31}H_{46}O_2$ requires 82·2; H, 10·7%) and a benzoate (benzoyl chloride Py), m.p. 138°; $[\alpha]_D$ −13° (*c*, 2·06) (Found: 84·1; H, 9·9. $C_{36}H_{50}O_2$ requires C, 84·1; H, %).

Dihydroenhydrin (VI) — A solution of enhydrin g) in EtOH (200 ml) was hydrogenated at spheric pressure over 10% Pd/C (1 g) until the rption of 80 ml of hydrogen. The catalyst filtered off, the solvent removed under reduced ssure and the residue crystallized from $CHCl_3$-ne to give colourless needles (VI; 1·7 g), m.p. , $[\alpha]_D$ −38° (*c*, 1·72); λ_{max} 210 nm (log ϵ, 4·11); (nujol), 1778, 1745, 1720, 1700, 1290, 1255, 0, 1170, 1140, 1075, 1025, 990, 960, 935, 915, 835, 825, 770 and 755 cm^{-1}; mass spectrum: 466 (M^+), 451, 435, 423, 407, 396, 374, 351, 258, 231, 203, 181, 175, 128 (Found: C, 59·1; 6·5. $C_{23}H_{30}O_{10}$ requires C, 59·2; H, 6·5%). rogenation of enhydrin in AcOH using PtO_2 as lyst also gave dihydroenhydrin.

action of enhydrin with diazomethane: *Formation pyrazoline* (V) — To a suspension of enhydrin mg) in ether (60 ml) an ethereal solution of N_2 was added. It was left at 0° overnight when rless cubes had deposited. These were collected crystallized from methylene chloride-ether to the pyrazoline (V; 135 mg), m.p. 245° (sintering 30°); λ_{max} 211 nm (log ϵ, 4·05) (Found: C, H, 6·0; N, 5·8. $C_{24}H_{30}N_2O_{10}$ requires C, 56·9; 0; N, 5·5%).

Alkali consumption by enhydrin — A solution of drin (100 mg) in MeOH (2 ml) and 1*N* NaOH ml) was heated on a water-bath at 75° for in. The solvent was removed under reduced ure and the residue diluted to 100 ml with ed water. Titration with 0·01*N* HCl indi- that 3·8 mol. equivalents of alkali had been med.

paration of chlorohydrin (VII) — To a solution ydrin (50 mg) in MeOH (2 ml) was added HCl (1 ml) at room temperature. The reaction re was heated at 60° for 5 min, left at room rature for 3 hr and then poured on crushed The precipitate was filtered off, washed with ter, dried and crystallized from CH_2Cl_2-hexane e colourless plates (VII; 35 mg), m.p. 219-20°; −48·6° (*c*, 1·02); IR (nujol), 3520, 1760, 1740, 1315, 1290, 1270, 1240, 1220, 1210, 1150, 1075, 1030, 995, 955, 870, 835, 810, 770 and m^{-1}. Mass spectrum: *m/e* 500 (M^+), 469, 441, 05, 377, 349, 348, 289, 275, 257, 256, 229 d: C, 55·3; H, 5·8. $C_{23}H_{29}ClO_{10}$ requires C, H, 5·8%).

paration of bromohydrin (VIII) — To a solu- enhydrin (100 mg) in MeOH (2 ml) 40% HBr (1 ml) was added and the solution warmed to 50° for 5 min. The solution was kept at room temperature for 3 hr when colourless plates separated. It was diluted with water, the precipitate collected, washed with water and the crude product (100 mg) crystallized twice from CH_2Cl_2-hexane (VIII; 90 mg), m.p. 214°; λ_{max} 211 nm (log ϵ, 3·28) (Found: C, 50·4; H, 5·3; Br, 14·1. $C_{23}H_{29}BrO_{10}$ requires C, 50·6; H, 5·3; Br, 14·6%).

Preparation of HCl addition product (IX) *of dihydroenhydrin* — A solution of dihydroenhydrin (120 mg) in 5% methanolic HCl (12 ml) was heated at 70° for 5 min and the clear solution kept at room temperature for 30 min. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was washed free of acid and dried (Na_2SO_4). The solvent was removed and the gummy residue chromatographed over a short column of silica gel and eluted with $CHCl_3$ containing 1% MeOH. This afforded a TLC homogeneous foam (Found: C, 54·7; H, 6·6. $C_{23}H_{31}ClO_{10}$ requires C, 54·9; H, 6·2%).

Reaction of $NaBH_4$ on enhydrin to give (a) *α,β-unsaturated ester* (XI) *and* (b) *dilactone* (XII) — (a) To a solution of enhydrin (456 mg) in MeOH (10 ml) was added dropwise with stirring at 0° a solution of $NaBH_4$ (380 mg) in MeOH (10 ml). The reaction mixture was stirred for further 1 hr. It was carefully neutralized using aq. 6% H_2SO_4, MeOH removed under reduced pressure, and the residue extracted with CH_2Cl_2. This gave a foam (200 mg). It was dissolved in $CHCl_3$ and chromatographed on a column of neutral alumina packed in $CHCl_3$. Eluates with $CHCl_3$ afforded a gummy residue, which on crystallization from CH_2Cl_2-hexane gave colourless needles (XI; 45 mg), m.p. 213°; λ_{max} 220 nm (log ϵ, 3·87); IR (nujol) 1780, 1740, 1700, 1300, 1260, 1150, 1130, 1080, 1045, 990, 940, 910, 875, 850, 840, 800, 755 and 720 cm^{-1}; mass spectrum: *m/e* 408 (M^+), 393, 376, 349, 335, 309, 293, 260, 233, 217, 205 (Found: C, 61·7; H, 7·1. $C_{21}H_{28}O_3$ requires C, 61·7; H, 6·9%).

(b) To a solution of enhydrin (500 mg) in MeOH (50 ml), small portions of $NaBH_4$ (500 mg) were added at room temperature. The reaction mixture was refluxed on a water-bath for 3 hr, allowed to cool and neutralized with aq. 6% H_2SO_4. The solvent was removed under reduced pressure and the residual gum extracted with CH_2Cl_2, washed with water and the organic layer dried (Na_2SO_4). Evaporation of the solvent gave a semicrystalline residue which after recrystallization from MeOH provided colourless needles (XII; 90 mg), m.p. 260°; λ_{max} 217 nm (log ϵ, 3·87); IR (nujol) 1780, 1750, 1640, 1420, 1320, 1260, 1230, 1202, 1188, 1155, 1125, 1085, 1072, 1050, 1020, 1000, 950, 920, 880, 840, 800, 780, 758 and 720 cm^{-1} (Found: C, 64·0; H, 6·8; M^+ 278. $C_{15}H_{18}O_5$ requires C, 64·7; H, 6·5%; mol. wt 278).

Alkaline hydrolysis of dihydroenhydrin to the diol (XIII) — A solution of dihydroenhydrin (580 mg) in MeOH (50 ml) and sodium methoxide (175 mg of sodium in 50 ml of MeOH) was kept at room temperature for 24 hr. The mixture was carefully neutralized with aq. 6% H_2SO_4 and evaporated to dryness under reduced pressure. The residue was extracted with $CHCl_3$ and the $CHCl_3$ extract (350 mg) crystallized from $CHCl_3$-hexane to give colourless needles (XIII; 200 mg), m.p. 210°; λ_{max} 213 nm

(log ϵ, 4·01); IR (nujol) 3570, 3480, 1750, 1700, 1670, 1620, 1310, 1260, 1230, 1170, 1125, 1110, 1070, 1050, 995, 980, 920, 865, 840, 820, 810, 775, 730 and 725 cm^{-1} (Found: C, 58·7; H, 7·0; M^+ 326. $C_{16}H_{22}O_7$ requires C, 58·9; H, 6·8%; mol. wt 326).

Acetylation of the diol (XIII) — The diol (XIII; 100 mg) was dissolved in pyridine (0·5 ml), Ac_2O (1·5 ml) and kept at room temperature for 14 hr. The reaction mixture was poured over crushed ice and the precipitate collected and crystallized from CH_2Cl_2-MeOH to give colourless cubes (XIV; 95 mg); m.p. 255-7°; λ_{max} 211 nm (log ϵ, 3·99); IR (nujol) 1778, 1740, 1700, 1620, 1320, 1280, 1225, 1170, 1100, 1090, 1030, 1000, 970, 960, 930, 900, 865, 845, 810, 775, 725 and 710 cm^{-1}; mass spectrum: *m/e* 410 (M^+), 351, 337, 291, 277, 259, 231, 203 (Found: C, 58·7; H, 6·8. $C_{20}H_{26}O_9$ requires C, 58·5; H, 6·4%).

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References

1. Desai, H. K., Gawad, D. H., Govindachari, T. R., Joshi, B. S., Kamat, V. N., Modi, J. D., Parthasarathy, P. C., Patankar, S. J., Sidhaye, A. R. & Viswanathan, N., *Indian J. Chem.*, **9** (1971), 611.
2. Joshi, B. S., Kamat, V. N. & Fuhrer, H., *Tetrahedron Lett.* (1971), 2373.
3. Chakravarti, R. N. & Dutta, A., *J. Indian chem. Soc.*, **29** (1952), 374.
4. Pakrashi, S. C., Ghosh Dastidar, P. P. & Gupta, S. K., *Phytochemistry*, **9** (1970), 459.
5. Krishnaswamy, N. R., Seshadri, T. R. & Vedantham, T. N. C., *Curr. Sci.*, **38** (1969), 591.
6. Krishnaswamy, N. R., Seshadri, T. R. & Vedantham, T. N. C., *Indian J. Chem.*, **8** (1970), 375.
7. Pakrashi, S. C. & Eshak Ali, *Indian J. Chem.*, **8** (1970), 569.
8. Pakrashi, S. C., Ghosh Dastidar, P. P. & Eshak Ali, *Indian J. Chem.*, **9** (1971), 84.
9. Bolger, L. M., Rees, H. H., Ghisalberti, E. L., Goad, L. J. & Goodwin, T. W., *Tetrahedron Lett.* (1970), 3043.
10. Sucrow, W. & Polyzou, P., *Tetrahedron Lett.* (1971), 1883.
11. Krishnaswamy, N. R., Seshadri, T. R. & Sharma, B. R., *Curr. Sci.*, **37** (1968), 94.
12. Krishnaswamy, N. R., Seshadri, T. R. & Vedantham, T. N. C., *Curr. Sci.*, **38** (1969), 284.
13. Herz, W. & Bhat, S. V., *J. org. Chem.*, **35** (1970), 2605.
14. Krishnaswamy, N. R., Seshadri, T. R. & Vedantham, T. N. C., *Curr. Sci.*, **40** (1971), 267.
15. Sorm, F. & Dolejs, L., *Guaianolides and germacranolides* (Hermann, Paris), 1965.
16. Waddell, T. G., Stocklin, W. & Geissman, T., *Tetrahedron Lett.* (1969), 1313; *Tetrahedron*, **26** (1970), 2397.
17. Samek, Z., *Tetrahedron Lett.* (1970), 671.
18. Benesova, V., Samek, Z., Herout, V. & Sorm, F., *Tetrahedron Lett.* (1970), 5017.