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TERPENOIDS—XXI*†

THE STRUCTURE OF COSTUNOLIDE, A NEW SESQUITERPENE LACTONE FROM COSTUS ROOT OIL

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Abstract—Costunolide, the new sesquiterpene lactone isolated from costus root oil (*Saussurea lappa* Clarke) is shown to have the structure I on the basis of dehydrogenation, ozonolyses and the formation of santanolide 'C' under mild conditions.

THE costus plant, *Saussurea lappa* Clarke belongs to the compositae family. The essential oil obtained from costus roots is highly valued in perfumery as a blending agent.[1] The chemistry of costus oil has been studied by a number of workers particularly Semmler and Feldstein[2] and more recently by Romanuk *et al.*[3] Recently we have isolated costus oil from costus roots under mild conditions by a modified solvent extraction procedure with petroleum ether, details of which will be published shortly. The oil thus obtained is quite different from the oil examined by previous workers. One of the constituents of this oil is a new crystalline sesquiterpene lactone which we have named costunolide. This lactone is rather unstable particularly at elevated temperature. This may explain why the earlier investigators failed to detect its presence in costus root oil. This appears to be a primary constituent of costus oil. Two preliminary communications[4,5] dealing with its structure and absolute configuration have already been published.

Costunolide (I) is a lactone having the molecular formula $C_{15}H_{20}O_2$. It contains three double bonds as shown by catalytic hydrogenation. Complete hydrogenation gives the fragrant liquid hexahydrocostunolide (II) of the formula $C_{15}H_{26}O_2$. Hence costunolide (I) is monocarbocyclic. Partial hydrogenation of costunolide gives dihydrocostunolide (III). Costunolide (I), dihydrocostunolide (III) and hexahydrocostunolide (II) show I.R. bands at 1764, 1779 and 1764 cm^{-1} respectively suggesting the presence of a γ-lactone.[6] While costunolide (I) gives 0·40 mole of formaldehyde on ozonolysis, dihydrocostunolide (III) gives only a trace on similar treatment. Costunolide (I) shows strong ultra-violet absorption (λ_{max} 213 mμ, log ε, 4·21) which is substantially reduced in dihydrocostunolide (III). Hence costunolide has a methylenic double bond ($=CH_2$) in conjugation with the carbonyl group of the lactone moiety. In agreement with this conclusion, costunolide consumes only two moles of

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* Part XX: Submitted to *Perf. & Ess. Oil Rec.*

† Contribution No. 615 from the National Chemical Laboratory, Poona, India.

[1] E. Guenther, *Essential Oils* 5, 446 (1952).

[2] F. W. Semmler and Feldstein, *Ber. Dtsch. Chem. Ges.* 47, 2433, 2687 (1914).

[3] M. Romanuk, V. Herout and F. Sorm, *Coll. Czech. Chem. Commun.* 23, 2188 (1958).

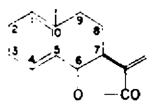
[4] A. Somasekar Rao, G. R. Kelkar and S. C. Bhattacharyya, *Chem. & Ind.* 1359 (1958).

[5] S. C. Bhattacharyya, G. R. Kelkar and A. Somasekar Rao, *Chem. & Ind.* 1069 (1959).

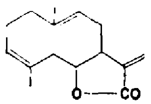
[6] L. J. Bellamy, *The Infra-red Spectra of Complex Molecules*, p. 153. Methuen, London (1954).

perbenzoic acid, the methylenic double bond being inert due to conjugation with the carbonyl group of lactone moiety.[7]

Lithium aluminium hydride reduction of costunolide (I) followed by selenium dehydrogenation of the crude diol (IV) gives 1,6-dimethyl naphthalene (V), presumably formed through initial cyclization of the diol (IV), followed by dehydrogenation with concomitant elimination of the C_3 unit, which in the parent compound necessarily will be the seat of the lactonic moiety. Hydrogenation of dihydrocostunolide in acetic acid gives rise to a crystalline solid lactone (VI) m.p. 154–155° which is bicyclic as shown by its molecular formula and fully saturated nature. This on selenium dehydrogenation gives 1-methyl-7-ethyl naphthalene (VII). These observations suggest that though costunolide (I) is monocarbocyclic it can be readily converted to compounds based on the bicyclodecane system. Rigorous proof of the carbon skeleton of lactone (VI) follows from these data.



I



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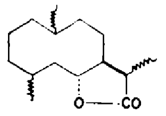

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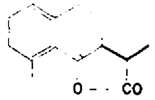

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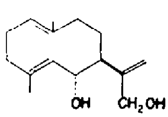

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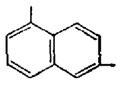

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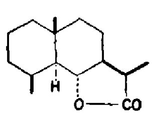

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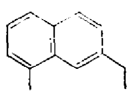

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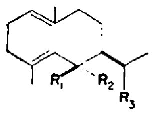
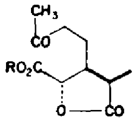


VIII $R_1 = -H, R_2 = -OH$
$R_3 = CH_2OH$

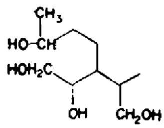
XI $R_1 = R_2 = -H$
$R_3 = -CO_2H$

XVI $R_1 = R_2 = -H$
$R_3 = -CO_2Me$

XVII $R_1 = R_2 = -H$
$R_3 = -CH_2OH$



IX R = –Me
XVIII R = –H



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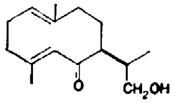

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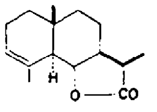
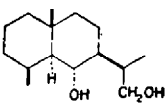


XIII



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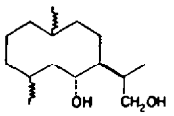
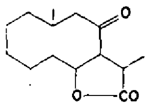


XV



XIX

Costunolide (I), dihydrocostunolide (III) and the diol (VIII) obtained by lithium aluminium hydride reduction of dihydrocostunolide (III) give levulic acid on ozonolysis. In view of this and the nature of the carbon skeleton of lactone (VI), and the absence of conjugation in dihydrocostunolide (III), costunolide must be represented by structure I or Ia. Ozonolysis of dihydrocostunolide (III), esterification of the resulting acids with diazomethane and fractionation gave as the higher boiling

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[7] Migrdichian, *Organic Synthesis* Vol. 2, p. 897. Reinhold, New York (1957).

portion, the compound IX having the molecular formula, $C_{11}H_{16}O_5$. On the basis of either formula I or Ia for costunolide, the compound IX must contain one ketogroup, one ester group and one γ-lactone group. In complete agreement with this expectation the I.R. spectrum of compound IX showed bands at 1718 (methyl ketone), 1756 (ester) and 1778 (γ-lactone) cm^{-1}. Compound IX forms a mono 2,4-dinitrophenylhydrazone, can be saponified to the acid XVIII of the formula $C_{10}H_{14}O_5$ and consumes 2 moles of hot alkali.

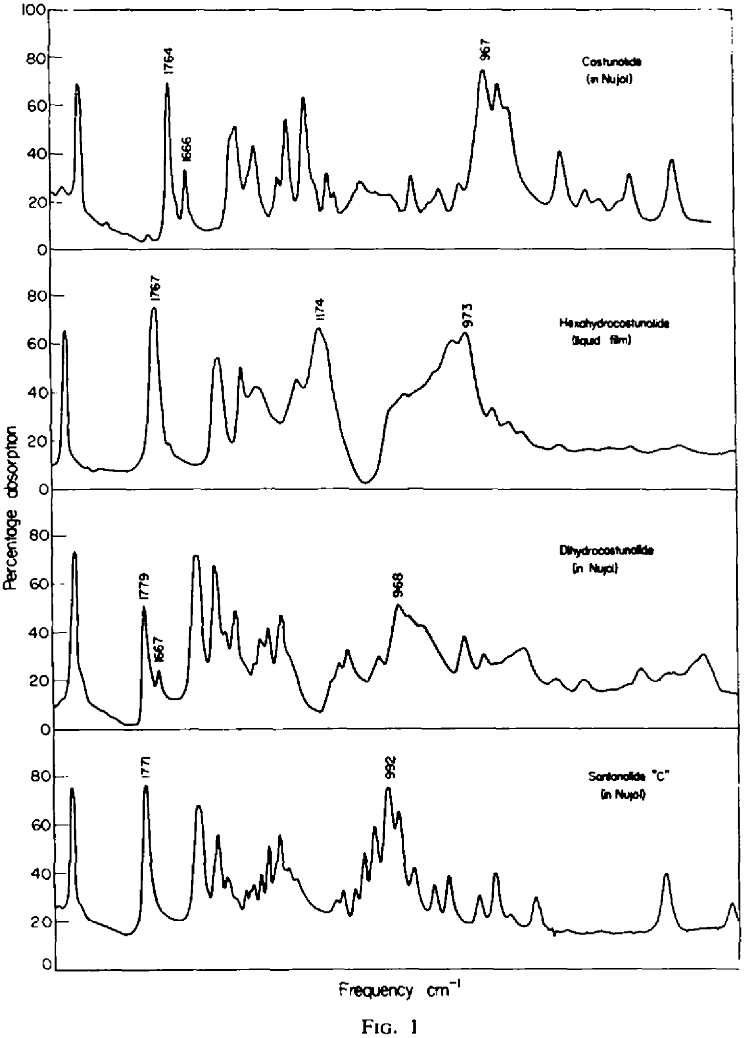

FIG. 1

Lithium aluminium hydride reduction of compound IX gives the tetrahydroxy compound X which reacts with sodium metaperiodate giving formaldehyde, thus showing the presence of two vicinal hydroxyl groups, in compound X. Costunolide undergoes hydrogenolysis during hydrogenation to give the acid XI. The diol VIII on oxidation with activated MnO_2 gives the α,β-unsaturated ketone XII. These observations show that costunolide has the structure shown in I.

The facile cyclization of some of the members of costunolide series also supports the presence of a ten membered carbocyclic ring in costunolide. The crystalline lactone (VI) is a cyclized product as mentioned earlier. Dihydrocostunolide (III) can be cyclized to the cyclodihydrocostunolide (XIII). The bicyclic nature of XIII is shown by the molecular formula and the presence of only one double bond. The absolute configuration of the lactone (VI) at C_8 has been found by the molecular rotation difference method.[8] The molecular rotation difference ($\Delta M_{\text{lactone-diol}} = +144{\cdot}8$) between the saturated lactone (VI) and the corresponding diol (XIV) shows that the lactone (VI) has the D-configuration at C_8. In the the region 700–1350 cm^{-1} the lactone (VI) shows an intense band at 992 cm^{-1} (Nujol) 994 cm^{-1} (chloroform) which is far more intense than any other band in this region. Hence VI is a trans-lactone.[9] This leads to the assignment of β-configuration to the C_7 side-chain of lactone VI. The absolute configuration of costunolide (I) follows from the absolute configuration of the lactone (VI) at C_8 and C_7. At an early stage of our investigations, we suspected the identity of the lactone (VI) m.p. 154–155° with santanolide 'C' (m.p. quoted at that time 137–139°).[10] However, in view of the large difference in m.p. and rotation we were hesitant to come to a definite conclusion; but in a recent paper Herout and Sorm[11] have stated that the previous sample of santanolide 'C' m.p. 137–139° was impure; the pure sample of santanolide 'C' subsequently prepared by them has a m.p. 153–154° which is the same as that of compound VI. In view of this, and the similarity of infra-red spectra, the identity of lactone (VI) with santanolide 'C' may be taken as established.

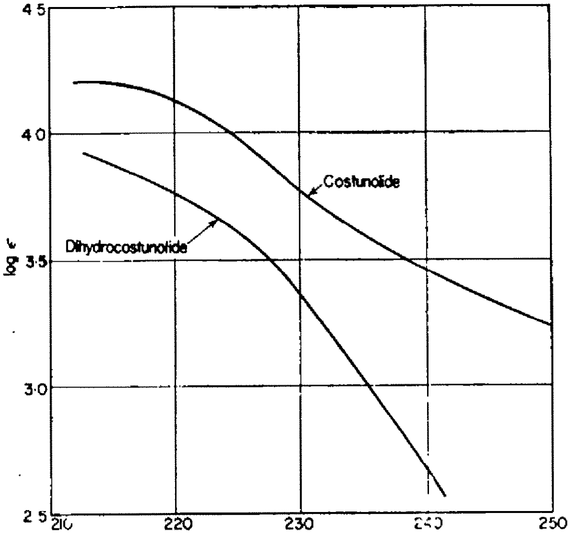

FIG. 2

The molecular rotation difference ($\Delta M_{\text{lactone-diol}} = -50$) between the hexahydrocostunolide (II) and the corresponding diol (XV) is negative whereas the

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[8] V. Sykora and M. Romanuk, *Coll. Czech. Chem. Comm.* **22**, 1909 (1957).

[9] T. Kanzawa, H. Kamio, M. Sumi and M. Nishikawa, *J. Amer. Chem. Soc.* **80**, 3705 (1958).

[10] O. Kovacs, V. Herout, M. Horak and F. Sorm, *Coll. Czech. Chem. Comm.* **21**, 225 (1956).

[11] V. Herout and F. Sorm, *Chem. & Ind.* 1067 (1959).

molecular rotation difference ($\Delta M_{\text{lactone-diol}} = +52$) is positive in the case of corresponding compounds prepared from arctiopicrin,[12] though the absolute configurations of costunolide and arctiopicrin[12] are identical at C_6 and C_7. This suggests that probably the two $C_{15}H_{26}O_2$ lactones do not have identical absolute configurations at both C_6 and C_7. Considering the method of preparation employed by us, hexahydrocostunolide (II) must have the same configurations as costunolide at C_6 and C_7. In the case of the $C_{15}H_{26}O_2$ lactone ($[\alpha]_D = +26°$) prepared from arctiopicrin a change in configuration at C_6 is rather unlikely whereas a change at C_7 may be possible, during the preparation of ethylene thioketal from the keto-lactone (XIX). The conversion of coprostane-4-one to the ethylene thioketal of cholestane-4-one may be cited as an analogy.[13] Hence in all probability the $C_{15}H_{26}O_2$ lactone derived from arctiopicrin is a *cis*-lactone having the C_7 side chain α-oriented.

The importance of ten-membered ring (carbocylic) compounds in terpene biogenesis has been discussed in the literature.[14,15] Costunolide is the parent lactone of this group. Recently, Herout and Sorm[11] have come to similar conclusions about the structure and absolute configuration of costunolide isolated from *Artemisia balchanorum*.

EXPERIMENTAL

All melting points and boiling points are uncorrected. Rotations were measured in chloroform. Ultra-violet spectra were taken in 95% alcohol. Microanalyses were carried out by Mr. Pansare and colleagues. Infra-red spectra were determined by Mrs. S. Das Gupta and Mr. Nagaraja Rao.

Isolation of costunolide (I)

Costus root oil obtained by modified solvent extraction method developed by us was diluted with four times its volume of petroleum ether (40–60°), cooled to 0°, seeded with costunolide and kept in the refrigerator for a week. The crystals (yield 15%) were filtered off and crystallized from methanol (3–4 ml. of methanol per gram of costunolide) avoiding excess heating. Needle shaped crystals were obtained. On heating they collapsed to a glassy mass at 106–107°. Rotation: $[\alpha]_D = +128°$ (c, 0·45).

U.V. spectrum: $\lambda_{\max}$ 213 mμ (log ε, 4·21) I.R. spectrum (in Nujol): Bands at 2833 1764, 1666, 1443, 1376, 1323, 1287, 1244, 1202, 1174, 1133, 1054, 1019, 994, 967, 952, 942, 893, 874, 863, 850, 841, 815 and 782 cm^{-1}. (Found: C, 77·89, H, 8·78; mol.wt. by saponification 236·1. $C_{15}H_{20}O_2$ requires: C, 77·55, H, 8·68%; mol.wt. 232·3).

Hexahydrocostunolide (II)

A solution of costunolide (10·9 g) in alcohol (250 ml) was hydrogenated at room temp at 60 atm in the presence of platinum oxide (0·14 g) catalyst. The temperature was raised to 40° towards the end. After the hydrogenation was complete the catalyst was filtered off and the solvent evaporated. The residue was passed through alumina and distilled to give hexahydrocostunolide (II) as a fragrant liquid b.p. 135–138°/0·4 mm, d^{30}_{30} 1·007, n^{30}_D 1·4880, $[\alpha]_D = -25°$ (c, 3·6). (Found: C, 75·5; H, 11·0. $C_{15}H_{26}O_2$ requires: C, 75·58; H, 11·0%). On chromatography a fraction with $[\alpha]_D = -36°$ was obtained.

I.R. spectrum (liquid film): bands at 2833, 1764, 1451, 1374, 1334, 1287, 1230, 1174, 1043, 1006. 988, 973, 945, 929, 919, 888, 835, 803 and 764 cm^{-1}

Hexahydrocostunolide (II) can also be obtained by the hydrogenation of costunolide (I) in alcohol in the presence of platinum catalyst at atm press; but the hydrogenation is slow after the absorption of 1 mole of hydrogen.

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[12] M. Suchy, M. Horak, V. Herout and F. Sorm, *Croatica Chem. Acta* **29**, 247 (1957); M. Suchy, V. Herout and F. Sorm, *Coll. Czech. Chem. Comm.* **24**, 1542 (1959).

[13] R. Stevenson and L. F. Fieser, *J. Amer. Chem. Soc.* **78**, 1409 (1956).

[14] D. H. R. Barton and P. de Mayo, *J. Chem. Soc.* 151 (1957).

[15] M. Suchy, M. Horak, V. Herout and F. Sorm, *Chem. & Ind.* **894** (1957); I. Ognjanoff, D. Ivanoff, V. Herout, M. Horak, J. Pliva and F. Sorm, *Ibid.* 820 (1957).

Dihydrocostunolide (III)

Costunolide (I, 10 g) in alcohol was hydrogenated using 5 % palladium-charcoal (0·5 g) as catalyst. The hydrogenation became extremely slow after the absorption of 1170 ml hydrogen. (temp 31°, press 710 mm), which corresponds to 1·03 moles hydrogen. The catalyst was filtered off and the alcohol removed under suction. The residue was taken up in ether, washed with sodium carbonate solution (to remove acidic products) and then with water. Removal of ether followed by crystallization of the residue from pet ether (40–60°) furnished 7·7 g dihydrocostunolide (III), m.p. 70–73°, which on recrystallization melted at 77–78°. Rotation: $[\alpha]_D = +113{\cdot}6°$ (*c*, 3·0). U.V. spectrum: no peak at 213 mμ. I.R. Spectrum (in Nujol): Bands at 2833, 1779, 1667, 1450, 1444, 1383, 1340, 1305, 1244, 1230, 1210, 1198, 1059, 1043, 994, 968, 952, 937, 884, 870, 850, 836, 811, 793, 758, and 719 cm^{-1}. (Found: C, 76·7; H, 9·48; $C_{15}H_{22}O_2$ requires: C, 76·88; H, 9·46 %).

The sodium carbonate extract on acidification furnished 0·35 g of the acid XI. The mother liquor left after crystallization of dihydrocostunolide (III) m.p. 77–78°, was a liquid which had $[\alpha]_D = -76°$ (*c*, 1·7). Probably this contained the C_{11} epimer of dihydrocostunolide (III). This was not further examined.

Santanolide "C" (VI)

Costunolide (I, 16·1 g) was hydrogenated in alcohol at atm press using platinum catalyst till the rate of hydrogenation became slow. The catalyst was filtered off and the solvent was removed under suction. The residue was dissolved in acetic acid and hydrogenated in the presence of platinum catalyst at room temp. Hydrogenation stopped after the absorption of nearly 2·8 moles hydrogen (1·2 moles in alcohol, 1·6 moles in acetic acid medium). The catalyst was filtered off and solvent removed at 100° using a water pump. The residue was taken in ether and washed with sodium carbonate solution and then with water. Removal of ether furnished 13 g residue.

This residue (20 g, from two batches) was dissolved in ether and cooled to −5°. The solid which separated was filtered off and recrystallized twice from petroleum ether (40–60°) to furnish santanolide 'C' (VI) as glistening plates m.p. 154–155° (yield 2·4 g). Rotation: $[\alpha]_D = +55{\cdot}1°$ (*c*, 3·5) lit[10,11] m.p. 137–139°; 153–154°. $[\alpha]_D = +92{\cdot}2°$.[10]

The lactone (VI) does not show strong absorption in the U.V. region, it does not react with perbenzoic acid; in the I.R., there is no band in the region around 1650 cm^{-1}; all these data go to show the absence of a double bond in lactone (VI). The lactone (VI) gradually dissolves in hot alkali and can be recovered unchanged from the alkaline solution on acidification.

I.R. spectrum (Nujol): bands at 2865, 1771, 1464, 1452, 1380, 1345, 1282, 1262, 1241, 1220, 1193, 1169, 1075, 1062, 1041, 1030, 1014, 992, 977, 958, 935, 918, 887, 870, 858, 835, 748 and 713 cm^{-1}. For comparison with santanolide 'C'[10] the I.R. spectrum was also determined in chloroform solution and found to be almost identical with that of santanolide 'C'.[10] (Found: C, 76·33; H, 10·10; $C_{15}H_{24}O_2$ requires: C, 76·22, H, 10·24 %).

The mother liquor left after the crystallization of lactone VI on chromatography gave the hexahydrocostunolide (II) as a liquid b.p. 160°/1·5 mm (bath, $[\alpha]_D = -25°$ (*c*, 3·5). I.R. spectrum was identical with that of hexahydrocostunolide (II) obtained by previous method.

Isolation of the acid (XI)

Costunolide (10 g) was hydrogenated in alcohol using 5 % palladium charcoal as catalyst. The hydrogenation was stopped after the absorption of 1·2 moles hydrogen. The catalyst was filtered off and the solvent removed. The residue was taken in ether and shaken with sodium bicarbonate solution. The sodium bicarbonate extract on acidification followed by extraction with ether and removal of solvent furnished 1·5 g of the crude acid (XI). This was esterified with diazomethane in the usual way to give the methyl ester (XVI) b.p. 130°/0·6 mm. (bath) (Found: C, 76·5; H, 9·9. $C_{16}H_{26}O_2$ requires: C, 76·75; H, 10·47 %).

The methyl ester (XVI) on lithium aluminium hydride reduction in the usual way (see below for a representative example) furnished the alcohol (XVII) as a liquid b.p. 135–140°/0·05 mm (bath). (Found: C, 80·6; H, 11·9; $C_{15}H_{26}O$ requires: C, 81·02; H, 11·79 %).

Diol (IV)

Costunolide (7·0 g) dissolved in ether (150 ml) was added slowly to a suspension of lithium aluminium hydride (1·2 g) in 100 ml ether at 0°. After stirring at 0° for 1 hr, and heating to reflux for 1½ hr, the reaction mixture was cooled, and the excess of lithium aluminium hydride decomposed carefully by adding dropwise cold water. The aluminium hydroxide was filtered off and washed thoroughly with ether. The ether layer was washed with water. Removal of solvent gave the diol IV (7·0 g) as a glassy material. A small portion was distilled to give slightly impure diol (IV) b.p. 160–180°/10^{-3} mm (bath). (Found: C, 74·98; H, 10·07; $C_{15}H_{24}O_2$ requires: C, 76·22; H, 10·24%).

Selenium dehydrogenation of the diol (IV)

The crude diol (IV, 6·6 g) was mixed with selenium (7·1 g) and heated in nitrogen atmosphere at 300° for 48 hr. Distillation furnished 1·00 g distillate (V) b.p. 128–135°/9 mm which was purified by distillation over sodium. The clear distillate thus obtained was characterized as 1,6-dimethylnaphthalene through (i) picrate (crystallized from alcohol), m.p. and mixed m.p. with an authentic sample 111°, lit.[14] m.p. 110–111°. (Found: C, 55·63; H, 3·67; N, 11·3. $C_{18}H_{15}N_3O_7$ requires: C, 56·1; H, 3·92, N, 10·91%), (ii) s-trinitrobenzene complex (crystallized from alcohol), m.p. and mixed m.p. with an authentic sample 130–131°, lit[16] m.p. 131–132°. (Found: C, 59·38; H, 3·63, N, 11·8. $C_{18}H_{15}N_3O_6$ requires: C, 58·53; H, 4·09; N, 11·38%).

Selenium dehydrogenation of the lactone (VI)

The lactone (VI, 1·2 g) was heated with selenium (1·6 g) at 270–300° for 48 hr in nitrogen atmosphere. The product was distilled off at 2 mm (bath temp 200° towards the end). To remove the lactonic impurities the distillate was heated on a steam bath for 1½ hr with an aqueous alcoholic solution of potassium hydroxide (1.2 g potassium hydroxide, 10 ml water and 2 ml alcohol). It was then diluted with water and extracted with ether. The ether extract on solvent removal, followed by chromatography of the residue (0·20 g) on Al_2O_3 (grade I, 10 g) furnished a clear liquid (0·16 g) which gave a s-trinitrobenzene complex (from alcohol) m.p. 99–102°. After two recrystallizations the m.p. rose to 107–108° alone or when mixed with an authentic sample of s-trinitrobenzene complex of 1-methyl-7-ethylnaphthalene prepared from hexahydrosantonin. (Found: C, 60·02; H, 4·79; N, 11·4 (for the sample from lactone (VI); C, 60·32; H, 4·38; N, 11·2 (for the sample from hexahydrosantonin); $C_{19}H_{17}N_3O_6$ requires; C, 59·53; H, 4·47; N, 10·96%).

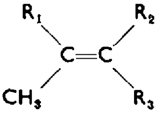
Ozonolysis

(i) *Estimation of formaldehyde*

Costunolide (I, 0·26 g) was dissolved in chloroform (75ml) cooled to 0° and a stream of ozonized oxygen (0·3 g of ozone per hr) was passed for 3 hr. The ozonized solution was steam distilled treated with dimedone solution and the chloroform removed by distillation. The formaldimethone m.p. 180–186° which separated weighed 0·132 g (0·40 moles). After one crystallization the sample of formaldimethone melted alone or on admixture with an authentic sample at 190–191°.

Dihydrocostunolide (III, 0·30 g) under similar conditions gave only 12 mg precipitate m.p. 176–184°.

(ii) *Ozonolysis of costunolide* (I) *and the estimation of the group*



Costunolide (I, 9·4 g) was dissolved in ethyl acetate, cooled to 0° and a stream of ozonized oxygen was passed till the ozonolysis was complete. The solvent was removed at 50° (towards the end the pressure was 2 mm). The residue was carefully heated with water on a water bath, for 2 hr. A clear solution was obtained. This was made up to 150 ml; 0·5 ml of the aqueous solution was taken and the number of —CO·CH_3 groups estimated by the consumption of iodine in alkaline medium[17] (Found: number of methyl ketone groups = 2·3 calculated for the structure (I) = 2·0). The higher value found experimentally may be due to the presence of aldehydes.

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[16] C. F. Seidel, P. H. Muller and H. Schinz, *Helv. Chem. Acta* **27**, 738 (1944).

[17] Scott, *Standard methods of Chemical Analysis Vol.* 2, p. 2136. Van Nostrand, New York (1939).

The remaining part of the aqueous solution was separated in the usual way to the following fractions: (A), neutral, volatile; (B) neutral, non-volatile; (C) volatile acids; (D) non-volatile acids. Fractions A and B were not studied. The presence of formic acid in fraction C was shown by chromotropic acid test.[18] Fraction D was esterified with diazomethane and fractionated to give three fractions: (i) b.p. 88–91°/15 mm (1·68 g); (ii) b.p. 60–80°/3 mm (0·75 g); (iii) b.p. 125–150°/3·5 mm (0·62 g). Fraction (i) $n_D^{35} = 1{\cdot}4164$ (Found: C, 54·84; H, 7·67; $C_6H_{10}O_3$ requires: C, 55·4; H, 7·7%) gave a 2,4 dinitro phenylhydrazone m.p. and mixed m.p. with an authentic sample of methyl levulinate 2,4-dinitro-phenylhydrazone 140–141° (Found: C, 46·30; H, 4·31, $C_{12}H_{14}N_4O_6$ requires: C, 46·45; H, 4·55%). Hence fraction (i) is identified as methyl levulinate.[19]

Fractions (ii) and (iii) were not studied critically.

Diol (VIII). Dihydrocostunolide (III, 9·0 g) was reduced with lithium aluminium hydride (3·0 g) in ether (200 ml) in the usual way. After the reaction was complete the mixture was cooled and the excess of lithium aluminium hydride decomposed with ice water. The aluminium hydroxide was filtered off and washed with ether. The ether layer on solvent removal gave the diol (VIII) $[\alpha]_D = +35{\cdot}1°$ as a syrup. This was used as such for the next step. I.R. spectrum (liquid film) no band at 1779 cm^{-1} (absence of γ-lactone), strong band at 3315 cm^{-1} (hydroxyl group).

(iii) *Ozonolysis of the diol* (VIII)

The diol (VIII, 6·2 g) was ozonized and worked up in the same way as mentioned earlier. Methyl levulinate (yield 1·3 g) was identified through its 2,4-dinitrophenylhydrazone m.p. and mixed m.p. with an authentic sample 140–141° (Found: N, 18·3%; $C_{12}H_{14}N_4O_6$ requires: N, 18·06%). The other fractions were not studied critically.

(iv) *Ozonolysis of dihydrocostunolide* (III)

Dihydrocostunolide (III, 17·6 g) was ozonized in ethyl acetate solution. It was worked up in the usual way. The acidic portion weighed 19·6 g. This was esterified with diazomethane and fractionated when two fractions were obtained. Fraction (i) b.p. 70–84/4·5 mm (mostly at 70–72°), (Yield 5·9 g) has been identified as methyl levulinate. Fraction (ii) b.p. 130–160°/0·4 mm (wt 10·3 g) was refractionated and the portion (IX) b.p. 180–185°/3 mm was analyzed. n_D^{26} 1·4620. Rotation: $[\alpha]_D^{28} = 0°$ (*c*, 5·0). (Found: C, 57·7; H, 7·2. Eq. wt. by saponification 113. $C_{11}H_{16}O_5$ requires: C, 57·88; H, 7·07%. Eq. wt. 114).

Compound IX gave a yellow 2,4-dinitrophenylhydrazone m.p. 94–95° (Found: N, 13·6. $C_{17}H_{20}O_8N_4$ requires: N, 13·72%).

Saponification of ester (IX). The ester (IX, 1·4 g) was heated for 2 hr on a water bath with an alcoholic solution of potassium hydroxide (1·3 g potassium hydroxide, 2·5 ml water, 10 ml alcohol). The saponified solution was diluted and extracted with chloroform to remove unsaponified ester (IX). The aqueous layer was made acidic and heated on a water bath for 1 hr. It was then extracted with chloroform in a liquid–liquid extractor. The chloroform extract on solvent removal followed by distillation of the residue furnished the acid (XVIII), b.p. 150–170°/0·002 mm (bath, 1·2 g) (Found: C, 55·6; H, 6·6. $C_{10}H_{14}O_5$ requires: C, 56·07; H, 6·59%).

Lithium aluminium hydride reduction of ester (IX).

The ester (IX, 0·5 g) was reduced by lithium aluminium hydride (0·5 g) suspended in ether (30 ml, 1½ hr at 0°; 5 hr at 45°). After the reduction was complete the excess lithium aluminium hydride was decomposed carefully with cold water. Enough dilute sulphuric acid (2 N) was added to dissolve the precipitate. The aqueous solution was extracted twice with ether and the ether extracts rejected. The aqueous layer which contained the tetrahydroxy compound (X) was neutralized with sodium carbonate solution and the precipitate obtained was filtered off. The filtrate was concentrated to a small volume and extracted with chloroform for 24 hr in a liquid–liquid extractor. The chloroform extract on solvent removal gave 0·4 g residue which was dissolved in water, filtered and the filtrate concentrated in vacuum. The tetrahydroxy compound (X) thus obtained did not crystallize. On treatment with

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[18] Feigl, *Spot tests in Organic Analysis* p. 340. Elsevier, New York (1956).

[19] Heilbron and Bunbury *Dictionary of Organic Compounds* Vol. 3, p. 178. Eyre and Spottiswoode, London (1953).

sodium metaperiodate solution the crude tetrahydroxy compound (X) gave formaldehyde (estimated as formaldimethone) in nearly quantitative yield.

Lithium aluminium hydride reduction of the lactone (VI). The lactone (VI) was reduced by lithium aluminium hydride in the usual way. The diol (XIV) was obtained as needle shaped crystals m.p. 116–117° (crystallized from benzene–petroleum ether mixture). Rotation: $[\alpha]_D^{27} = -6{\cdot}2$ (c, 4·8). (Found: C, 74·95; H, 11·5. $C_{15}H_{28}O_2$ requires: C, 74·95; H, 11·74%).

I.R. spectrum (Nujol): Bands at 3110, 2820, 1452, 1374, 1341, 1261, 1053, 1040, 1019, 1002, 974, 961, 945, 917 892, 869 ,853 and 832 cm^{-1}.

Diol (XV). Hexahydrocostunolide (II, 2·2 g) ($[\alpha]_D = -32°$) (purified by chromatography) was reduced with lithium aluminium hydride (0·5 g) in the usual way to give the diol (XV), b.p. 153–155°/ 0·4 mm (evaporative distillation). $[\alpha]_D = -11°$ (c, 2·1). (Found: C, 74·03; H, 11·95. $C_{15}H_{30}O_2$ requires: C, 74·32; H, 12·48%). I.R. spectrum: No band at 1764 cm^{-1} (absence of γ-lactone). Strong band at 3310 cm^{-1} (hydroxyl group).

Cyclodihydrocostunolide (XIII). Dihydrocostunolide (III, 0·15 g) was heated to reflux with acetic acid (1 ml) and acetic anhydride (1 ml) for 4 hr. The solvent was removed under suction. The residue was taken up in ether and washed with sodium carbonate solution. The ether extract on solvent removal followed by short-path distillation in vacuum (1 mm) gave 0·13 g cyclodihydrocostunolide (XIII) which had the m.p. 120–122° after two crystallizations from pet ether (Found: C, 77·4; H, 9·4. $C_{15}H_{22}O_2$ requires: C, 76·88; H, 9·46%). The compound (XIII) after 8 crystallizations showed a m.p. 134–136° lit[20,11] m.p. 136–7°; 135·5.

The α,β-unsaturated ketone (XII). The diol (VIII, 0·15 g) was dissolved in pet ether (40–60°) and shaken with activated MnO_2 at room temp for 24 hr. The manganese dioxide was filtered off. The filtrate on removal of solvent at 40° gave a viscous oil which appears to be impure α,β-unsaturated ketone (XII). U.V. spectrum: λ_{max} 229 mμ; log ε 3·2. No attempt was made to isolate the pure α,β-unsaturated ketone (XII).

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[20] W. Cocker and T. B. H. McMurry, *J. Chem. Soc.* 4549 (1956).