Tetrahedron, 1963, Vol. 19, pp. 1377 to 1385. Pergamon Press Ltd. Printed in Northern Ireland

TERPENOIDS—I

OXIDATION OF RING A IN EUPHOL[1]

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(*Received* 16 *April* 1963)

Abstract—Euphadiene-3-one was autoxidized in potassium-t-butoxide to yield 2-hydroxy-1,8,24-euphatriene-3-one (diosphenol IIa) and 1-hydroxy-2-oxa-euphadiene-3-one (lactol IIIa) which was reduced to the δ-lactone IV and the hemiacetal Va. Hydrogenation and acetylation of the diosphenol IIa as well as hydrogenation of the diosphenol acetate IIb yielded the same 3-acetoxy-euphene-2-one VIIb. Direct acetoxylation of euphene-3-one formed 2α-acetoxy-euphene-3-one. From the autoxidation, the anhydride IX was isolated and characterized by synthesis.

IN THE course of studies with substances possessing a selective action on neoplastic cells, we have been interested in triterpenoids displaying anti-tumor activity. Certain Euphorbia species seem to have a limited anti-tumor activity as indicated by tests reported in the literature.[3] Screening tests performed for us on certain fractions obtained from the resin of *Euphorbia resinifera* resulted in preliminary promising results.[4] Furthermore, experiments have also been performed with several Euphorbia lattices in order to determine their tumor promoting action; they have been compared to croton oil obtained from Croton tiglium (Euphorbiaceae) and a similar action has been discovered with certain species.[5]

We have concentrated out attention on euphol, a major constituent of *Euphorbia resinifera*, and it was proposed to introduce certain groupings for further biological experimentation. The occurrence of an α-hydroxy ketone or of a diosphenol grouping in ring A of certain cucurbitacins seemed to be one of the attributes of their anti-tumor activity which has been extensively studied in our laboratory.[6] The present paper deals with the reactions encountered during the preparation of the oxygenated derivatives of euphol.

The crude ethanolic extract of commercial resin from *Euphorbia resinifera* yields upon extraction with hexane a white crystalline mixture from which pure euphol and euphorbol were obtained by chromatographic separation. Euphol was oxidized to

[1] Supported in part by a research grant CY-2810 from the National Cancer Institute of the National Institutes of Health, Public Health Service, U.S.

[2] This work was initiated at the Walker Laboratory of the Sloan-Kettering Institute for Cancer Research, Rye, N.Y. during the tenure of a visiting fellowship which is hereby acknowledged.

[3] M. Belkin and D. B. Fitzgerald, *J. Nat. Cancer Inst.* **13,** 139 (1952).

[4] Unpublished results, Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York.

[5] F. J. C. Roe and W. E. H. Peirce, *Cancer Res.* **21,** 338 (1961).

[6] R. Gallily, B. Shohat, J. Kalish, S. Gitter and D. Lavie, *Cancer Res.* **22,** 1038 (1962); B. Shohat, S. Gitter and D. Lavie, *Cancer Chemother. Reports* No. 23, 19 (1962).

euphadiene-3-one (I) which was used for our investigations. The introduction of an oxygen function α- to the C-3 carbonyl group was performed by shaking in oxygen a solution of euphadiene-3-one (I) in t-butanol saturated with potassium t-butoxide.[7,8] The first mole of oxygen which was rapidly absorbed produced the α-diketone derivative, it was found on chromatoplate (two spots) to consist of a mixture of the two tautomeric forms, the diosphenol (IIa) and the α-diketone (II); the ultraviolet (λ_{max} 269 mμ, ε = 7,900) as well as the I.R. (ν_{max} 1715, 1672 and 1653 cm^{-1}) spectra were in complete agreement with these assignments. Upon acetylation at room temperature a diosphenol acetate (IIb) was obtained, λ_{max} 236 mμ (ε = 9,000); ν_{max} 1764 cm^{-1}. The N.M.R. spectrum of the diosphenol [2-hydroxy-1,8,24-euphatriene-3-one] IIa showed a singlet at lower field due to the vinylic hydrogen at C-1, τ = 3·40 while in the acetylated product (IIb) this signal shifted to τ = 3·02.

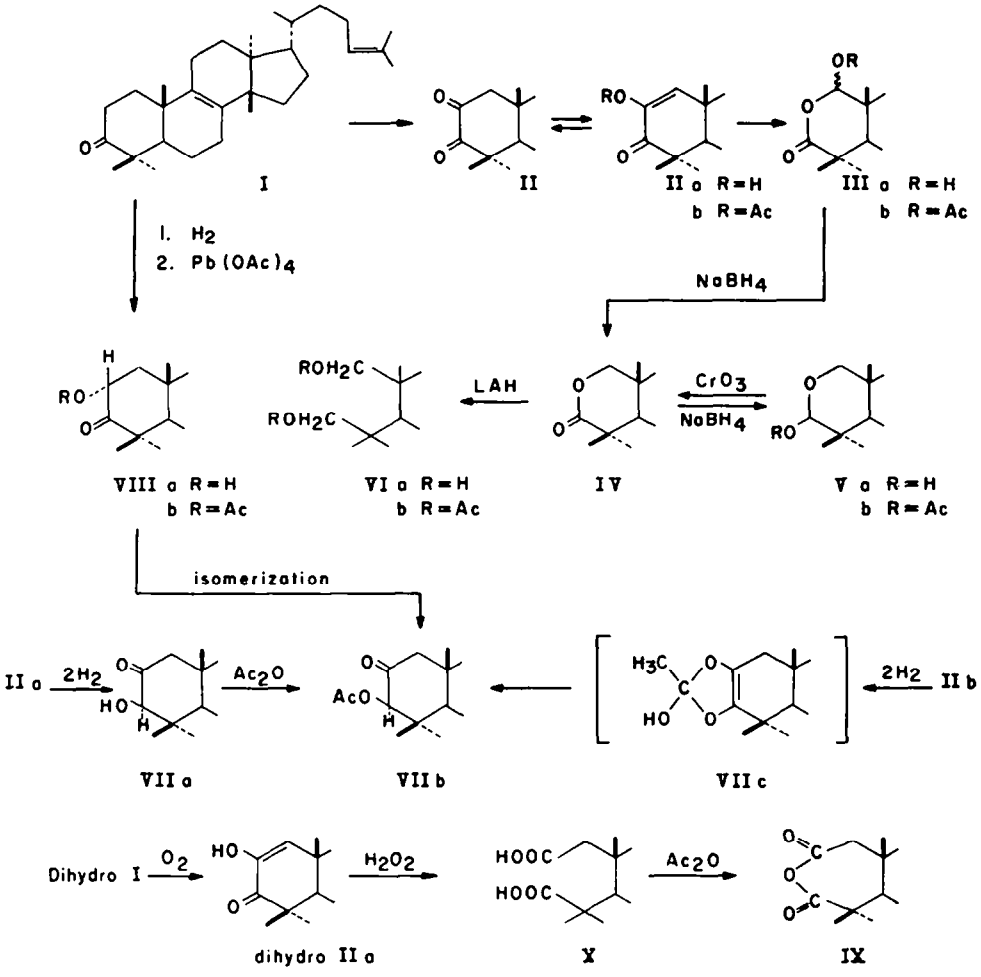
When, during the process of autoxidation, a second mole of oxygen was allowed to be absorbed, the product isolated from the reaction mixture was identified as the lactol IIIa (1-hydroxy-2-oxa-euphadiene-3-one), ν_{max} 1710 and 1107 cm^{-1}. The formation of such a lactol has already been described and was interpreted through the formation of a ring A seco-2-nor-aldehydo carboxylic acid which cyclizes upon acidification.[8] The loss of a carbon atom (C-2) during this process and the formation of the heterocyclic six membered ring is now shown unequivocally by the N.M.R. spectrum of the substances. Indeed a characteristic singlet related to the C-1 proton was found at τ = 4·40; this peak was not sharp due to coupling with the proton of the adjacent hydroxyl group, however upon acetylation, this signal in the lactol acetate (IIIb) was found to be shifted downfield to τ = 3·58 and appeared now as a sharp peak. From this observation it can be deduced that no protons are neighboring the C-1 hydrogen as would be expected from a lactol derived from a 2,3-seco-aldehydo-acid (i.e. a seven membered ring lactol). The actual elimination of a carbon atom (C-2) during the process of oxydation is thereby indicated.

The structure of the lactol (IIIa) was further attested by its reduction to the corresponding δ-lactone IV (2-oxa-euphadiene-3-one) using sodium borohydride in the presence of one mole of potassium hydroxide, ν_{max} 1739 cm^{-1}, no absorption in the OH region. However, in addition to the lactone, a second product was isolated from the reaction mixture in 45 per cent yield. This substance, which failed to crystallize, had no absorption in the carbonyl region of the I.R. spectrum, but bands were present at ν_{max} 3450 and 1068 cm^{-1} accounting for a hydroxyl group and an ether linkage. Upon acetylation at room temperature a crystalline monoacetate was obtained $C_{31}H_{50}O_3$ for which structure Vb (3-acetoxy-2-oxa-euphadiene) was assigned. The hemiacetal (Va) structure, which was proven unequivocally as follows, could have been formed by the direct reduction of the lactonic carbonyl group. Indeed when the lactone (IV) itself was subjected to the same sodium borohydride reduction procedure, it was entirely converted to the hemiacetal Va, which could be reconverted to the lactone IV by oxidation with chromium trioxide. The N.M.R. spectrum of Va displayed two doublets of an AB type centered at τ = 6·69 and 6·09 (J_{AB} = 11 cps) which are related to the two protons at C-1, while a broad peak, sharpening upon addition of CF_3COOH and located at τ = 5·66, is related to the C-3 proton adjacent

[7] E. J. Bailey, D. H. R. Barton, J. Elks and J. F. Templeton, *J. Chem. Soc.* 1578 (1962).
[8] R. Hanna and G. Ourisson, *Bull. Soc. Chim. Fr.* 1945 (1961).

to the hydroxyl group. In the monoacetate Vb this singlet was found shifted downfield, $\tau = 4{\cdot}71$. The chemical shift described here is indeed in good agreement with data reported for similar protons in a series of acetylated sugars.[9]

Reductions of lactones to hemiacetals with metal hydrides have been reported in several instances using lithium aluminum hydride;[10] with sodium borohydride, aldonic



acid lactones were reduced to aldoses[11a] and steroid ring B lactones, to the corresponding hemiacetals.[11b] On treating a similar lactol acetate in the 4,4-dimethylcholestenone series with sodium borohydride, Hanna and Ourisson reported[8] a product showing hydroxyl bands only, which was not further investigated; this product had probably

[9] R. U. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, *J. Amer. Chem. Soc.* **80**, 6098 (1958).

[10] W. Herz, W. A Rohde, K. Rabindran, P. Jayaraman and N. Viswanathan, *J. Amer. Chem. Soc.* **84**, 3857 (1962); A. W. Burgstahler and M. O. Abdel-Rahman, *Ibid.* **85**, 173 (1963).

[11(a)] M. L. Wolfrom and H. B. Wood, *J. Amer. Chem. Soc.* **73**, 2933 (1951).

[11(b)] N. W. Atwater, *J. Amer. Chem. Soc.* **83**, 3071 (1961).

also a hemiacetal structure. As expected, when the lactone IV was subjected to lithium aluminum hydride reduction, it was converted to the seco-A-2-nordiol (VIa) which upon acetylation yielded a diacetate (VIb). Unfortunately these two substances could not be induced to crystallize for complete characterization, however all the spectral evidences of the diacetate supported the proposed structure: ν_{max} 1724 and 1242 cm^{-1}; the N.M.R. spectrum of VIb displayed two series of AB patterns of lines related to the two $-CH_2OAc$: $\tau = 6{\cdot}22$ and $5{\cdot}97$ ($J_{AB} = 10{\cdot}9$ cps), and $\tau = 6{\cdot}03$ and $5{\cdot}70$ ($J_{AB} = 12{\cdot}8$ cps); such a pattern indicates the non-equivalence of the protons in the respective two groupings, and is probably due to restricted free rotation in the asymmetric molecule.

In order to prepare the α-hydroxy ketone in ring A, the diosphenol containing compound (IIa) was reduced catalytically over palladium on charcoal. The side chain double bond followed by the ring A enolic double bond were reduced during the process, yielding a hydroxy ketone derivative which would not crystallize, but was homogeneous on chromatoplate. Structure VIIa was assigned for this compound based on the following spectroscopical evidences: ν_{max} 1712 cm^{-1}; N.M.R., *singlet* at $\tau = 5{\cdot}95$ accounting for one hydrogen and two AB type doublets centered at $\tau = 7{\cdot}69$ and $7{\cdot}35$ ($J_{AB} = 12$ cps) accounting for two hydrogens. Upon acetylation of the hydroxy ketone a crystalline keto acetate was obtained: ν_{max} 1742 and 1730 cm^{-1}; N.M.R. spectrum, singlet at $\tau = 4{\cdot}95$ for one hydrogen and a broad peak at $\tau = 7{\cdot}50$ accounting for two hydrogens. These data can be rationalized in terms of structure VIIa for the hydroxy ketone and VIIb for its acetate derivative. In both substances the low field signals (singlets) are attributed to the C-3 H indicating no neighboring hydrogens, while the higher field signals are related to the two protons (C-1)α to the keto group. During hydrogenation, a rear attack from the less hindered side of the molecule is to be expected and therefore the hydroxyl group at C-2 should become β-axial-oriented.* Factors governing the conformation of ring A in 4,4-dimethyl steroids and triterpenes are the non bonded 1,3-homoannular interactions of the methyl groups at C-4 and C-10.[12] The additional two 1,3-diaxial interactions added by the introduction of the new C-2-axial substituent would result in a strain which is released by enolization of the carbonyl in the 2β-axial-hydroxy-3-keto system to the 2-keto-3β-equatorial arrangement VIIa. It is expected that such a mechanism would lead to the more stable β-equatorial configuration of the hydroxyl group at C-3 and indeed the I.R. spectrum of the carbonyl region of VIIb (ν_{max} 1742 and 1730 cm^{-1}) supported this assumption. Further demonstration of the instability of a C-2 β-axial substituent in such a system was obtained during the catalytic hydrogenation of the diosphenol acetate IIb; one product which was found identical to the C-3 acetate VIIb was isolated from this reaction. Migrations of acetoxy groups of a similar nature have been recorded in the literature in several cases in which the formation of a cyclic intermediate (VIIc) has been proposed.[13]

Alternatively the 2-acetoxy derivative was prepared by direct acetoxylation of euphene-3-one (24-dihydro-I) using lead tetracetate in acetic acid in the presence of

* An alternative possibility is a 1-4 addition during the hydrogenation.

[12] J. S. E. Holker, *Proc. Chem. Soc.* 464 (1961).

[13] R. Wenger, H. Dutler, H. Wehrli, K. Schaffner and O. Jeger, *Helv. Chim. Acta* **45**, 2420 (1962) and references cited therein.

boron trifluoride.[14] As expected, approach from the less hindered rear face of the molecule should lead to the 2-α-equatorial-acetoxy derivative VIIIb. Indeed, the carbonyl region of the I.R. spectrum indicated bands at 1742 and 1730 cm^{-1}. The N.M.R. spectrum of compound VIIIb supported the location of the acetoxy group at C-2 and its configuration. The proton at C-2 displayed a quartet of lines centered at $\tau = 4{\cdot}30$ ($J_{ae} = 6{\cdot}5$ cps and $J_{aa} = 13{\cdot}0$ cps). In contradistinction to compound VIIb, no signals were detected now in the region characteristic for protons α to a keto function. Upon hydrolysis in acidic conditions[15] the 2-α-equatorial hydroxy 3-keto derivative VIIIa was obtained, ν_{max} 1718 cm^{-1}.

Adsorption on active basic alumina converted the 2-α-equatorial-acetoxy ketone VIIIb into the isomer VIIb. In this case also, isomerization proceeds by enolization and acyl group migration probably through the cyclic intermediate (VIIc). The acetoxy ketone VIIb, which was obtained by the various methods described herewith is therefore thermodynamically the most stable derivative in this series. Although it is difficult to visualize on a model the favoring of the 3β-equatorial upon the 2α-equatorial orientation of the acetoxy group, this fact is probably connected with the twisted shape of ring A; this problem will be discussed extensively in a subsequent publication using N.M.R. measurements.

It is noteworthy that a substance which was identified as the anhydride IX was also formed during the autoxidation of euphadiene-3-one (I) to diosphenol IIa, it crystallized in minor quantities from the reaction mixture following the hydrogenation procedure. This anhydride structure was proven unambiguously through synthesis: euphene-3-one (24-dihydro-I) was oxidized by the above described potassium t-butoxide procedure to the corresponding α-diketone derivative (dihydro-IIa) which was then cleaved with alkaline hydrogen peroxide to the seco-2,3-dicarboxylic acid X. Heating the diacid X in acetic anhydride, yielded the same anhydride IX with the characteristic I.R. bands at ν_{max} 1802 and 1759; 1034 and 1014 cm^{-1}. A similar reaction has been described during the oxidation of dihydro-oxomanoyl oxide in which in addition to the product possessing the diosphenol grouping, a 2,3-seco-acid has been isolated from the reaction mixture.[16] The formation of the anhydride as by-product is readily explained by the initial formation of an α-hydroperoxy-ketone and its cleavage to seco-2-aldehydo-3-carboxylic acid, either by a four membered ring intermediate mechanism proposed by Doering and Haines[17] or by an alternate peroxide intermediate based on Criegee's mechanism.[18] The aldehyde, under the basic reaction conditions, is subsequently oxidized to a carboxylic group thus forming the 2,3-seco-dicarboxylic acid which upon cylization forms the anhydride IX.

EXPERIMENTAL

M.p.s were taken on a Kofler hot-stage microscope and are corrected. All optical rotation measurements were carried out in chloroform solution. U.V. absorption spectra were done on a Cary 14 Spectrophotometer in ethanol solution. I.R. spectra were recorded on a Perkin Elmer Infracord model 137 spectrometer equipped with a sodium chloride prism and, unless otherwise

[14] H. B. Henbest, D. N. Jones and G. P. Slater, *J. Chem. Soc.* 4472 (1961).

[15] Alkaline treatment resulted in a complex intractable mixture, however acidic treatment led to the formation of some isoeuphol derivative which was separated by chromatography.

[16] P. K. Grant and R. M. Carman, *J. Chem. Soc.* 3740 (1962).

[17] W. von Doering and R. M. Haines, *J. Amer. Chem. Soc.* **76**, 482 (1954).

[18] E. Elkik, *Bull. Soc. Chim. Fr.* 933 (1959).

stated, were determined in chloroform solution in 5–10% concentration. N.M.R. spectra were recorded on a Varian A-60 Spectrometer. The spectra were determined in deuterated chloroform solutions of about 5–10% concentration and containing tetramethyl silane as internal standard. Thin layer chromatography was done on chromatoplates of silica gel G (Merck) and spots were developed with potassium permanganate 0·5% solution in a saturated cupric acetate solution.

Isolation of euphol

Dry latex of *Euphorbia resinifera* (200 g) was left over a period of 24 hr with occasional shaking in ethyl alcohol (1·0 l.). The solution was filtered, concentrated to ½ volume and extracted several times with hexane. The combined extracts were then washed with water, dried over sodium sulfate and concentrated to a small volume, crystallization took place upon cooling. The collected crystalline product (20 g) consisted of a mixture mainly of euphol and euphorbol which was chromatographed through a column packed with Florisil (100–200 mesh). Elution with hexane benzene (1:1) afforded euphol, followed by a mixture of euphol and euphorbol. The euphol fractions were combined yielding 7·5 g of a rather pure product which was used as such in the following reactions. For complete characterization a sample was crystallized several times from acetone.

Oxidation of euphol to euphadiene-3-one (I)

To an ice cold solution of euphol (5 g) in acetone (300 ml, distilled over potassium permanganate), 4 ml of a solution of chromium trioxide (27 g CrO_3 in 100 ml of 35% aqueous solution of H_2SO_4) was added dropwise with stirring. The reaction mixture was continuously stirred at 5–10° for an additional ½ hr when the excess oxidant was destroyed with methanol. The solution was concentrated to ¼ volume and poured into water. The solid material was collected, washed thoroughly with water, dried and chromatographed on Florisil. Elution with hexane benzene (4:1) yielded 4·5 g ketone I which was crystallized from ethanol m.p. 118°, $[\alpha]_D$ +73°, ν_{max} 1703 cm^{-1}. It was found identical in all respects with the known euphadiene-3-one obtained by oxidation with CrO_3 in acetic acid.[19]

2-*Hydroxy*-1,8,24-*euphatriene*-3-*one* (*diosphenol* IIa)

A solution of euphadiene-3-one (1 g) in 25 ml dry t-butanol was added to a saturated solution (40 ml) potassium t-butoxide in t-butanol and shaken in oxygen (atm. press.) until one equivalent of oxygen was absorbed (15–20 min). The reaction mixture was poured into ice, acidified with dil. hydrochloric acid to pH 3 and extracted with benzene. The benzene extract was washed twice with water, then dried (Na_2SO_4) and concentrated *in vacuo* to a yellowish glassy residue which was crystallized several times from methanol, yielding 650 mg of the diosphenol IIa, m.p. 93–95°, $[\alpha]_D$ +38·8° (*c*, 2·01), positive $FeCl_3$ test, ν_{max} 1715 (weak) 1672, 1653 and 1404 cm^{-1}; λ_{max} 269 mμ (ε 7,900), $\lambda_{max}^{1\%\,KOH}$ 313 mμ (ε 5,300), λ_{max}^{HCl} 269 mμ (ε 8,100). (Found: C, 82·04; H, 10·47. $C_{30}H_{48}O_2$ requires: C, 82·13; H, 10·57%). Two spots on chromatoplate (benzene system), an upper large spot (R_f 0·66) which could be developed either by $KMnO_4$ or by $FeCl_3$ solution and a lower small spot (R_f 0·52) which was not coloured by $FeCl_3$. When the benzene solution of crude diosphenol was left overnight, the product obtained was found to be a mixture of about ⅓ of the diketone (ν_{max} 1715) and ⅔ of the diosphenol tautomer.

2-*Acetoxy*-1,8,24-*euphatriene*-3-*one* (IIb)

Acetylation of the diosphenol IIa with an acetic anhydride pyridine mixture, overnight at room temp, resulted in the formation of the diosphenol acetate (IIb) which could not be induced to crystallize, $[\alpha]_D$ +14·2° (*c*, 3·80), ν_{max} 1764, 1684, 1647 (shoulder) and 1210 (broad) cm^{-1}; λ_{max} 236 mμ (ε 9,000), after 24 hr $\lambda_{max}^{1\%KOH}$ 313 mμ, λ_{max}^{HCl} 269 mμ. On chromatoplate it displayed one round spot only, both original spots of the starting product having disappeared.

1-*Hydroxy*-2-*oxa*-*euphadiene*-3-*one* (*lactol* IIIa)

Euphadiene-3-one (1 g) was allowed to absorb two equivalents of oxygen under the same experimental conditions described above. The second mole of oxygen was consumed in about 40 hr. The reaction mixture, following a similar work up, yielded a white foam which crystallized from aqueous

[19] M. Vilkas, G. Dupont and R. Dulou, *Bull. Soc. Chim. Fr.* 809 (1949).

ethanol to the lactol IIIa, m.p. 148–50°, $[\alpha]_D$ +47·7° (*c*, 1·11), negative $FeCl_3$ test, ν_{max}^{KBr} 1710, 1107, 1000 cm^{-1}; U.V. end absorption only. (Found: C, 78·49; H, 10·46. $C_{29}H_{46}O_3$ requires: C, 78·68; H, 10·47%). The same lactol could also be obtained by oxidation of the diosphenol with one equivalent of oxygen by the same procedure.

The lactol (IIIa; 200 mg) was acetylated with acetic anhydride and pyridine, overnight at room temp. The crude acetate IIIb crystallized from ethanol, m.p. 126–127°, $[\alpha]_D$ +71·3° (*c*, 1·74), ν_{max}^{KBr} 1770 (shoulder), 1757, 1232 (shoulder) 1214, 1199. (Found: C, 76·57; H, 9·87. $C_{31}H_{48}O_4$ requires: C, 76·81; H, 9·98%).

2-Oxa-euphadiene-3-one (IV) *and 3-hydroxy-2-oxa-euphadiene* (Va)

To a solution of the lactol (1 g) in methanol (100 ml) a methanolic solution of potassium hydroxide (one equivalent) and sodium borohydride (1 g) was added and stirred overnight at room temp. The reaction mixture was then acidified with dil. hydrochloric acid to pH 3, poured into ice and extracted with benzene. The extract was washed with water, dried (Na_2SO_4) and evaporated *in vacuo*, leaving a semicrystalline residue which was resolved into two spots on chromatoplate (benzene–ethyl acetate system). The crude product (950 mg) was chromatographed through acid washed alumina (Merck). A crystalline product (400 mg) was eluted with hexane benzene (1:1) and recrystallized from ethanol, m.p. 130–132°, $[\alpha]_D$ +35·0° (*c*, 0·97), ν_{max}^{KBr} 1739 and 1121 cm^{-1}. (Found: C, 81·59; H, 10·99. $C_{29}H_{46}O_2$ requires: C, 81·63; H, 10·87%). The product was assigned the lactone structure IV, *2-oxa-euphadiene-3-one*.

The product exhibiting the lower spot on chromatoplate was eluted with benzene (450 mg), it failed to crystallize, though homogeneous on chromatoplate, $[\alpha]_D$ −1·5° (*c*, 2·61), ν_{max} 3450, 1068 and 961 cm^{-1}. This compound was assigned the hemiacetal structure Va, *3-hydroxy-2-oxa-euphadiene*.

When the sodium borohydride reduction was performed without adding alkali, the main reaction product was the lactone, and only about 10% of hemiacetal could be isolated following chromatography.

3-Acetoxy-2-oxa-euphadiene (Vb)

The hemiacetal Va (200 mg) was acetylated overnight at room temp with acetic anhydride and pyridine; the monoacetate crystallized from ethanol, m.p. 115–116°, $[\alpha]_D$ +8·1° (*c*, 1·11), ν_{max}^{KBr} 1757, 1224, 1089, 1059 and 1041 cm^{-1}. (Found: C, 78·96; H, 10·60. $C_{31}H_{50}O_3$ requires: C, 79·10; H, 10·71%).

Oxidation of the hemiacetal Va *to the lactone* IV

The hemiacetal (100 mg) in acetone (20 ml, previously distilled over $KMnO_4$) was oxidized at 0° with a slight excess of CrO_3 (in sulfuric acid solution as indicated above). The excess oxidant was destroyed with methanol and the organic solvents were evaporated to a small volume; water was then added and the mixture was shaken with chloroform. The chloroform extract was washed with water, dried (Na_2SO_4) and distilled *in vacuo* leaving a crystalline residue (95 mg). Recrystallized from ethanol, yielding a compound identical in all respects with the lactone IV.

Sodium borohydride reduction of the lactone IV *to the hemiacetal* Va

Alternatively, when the lactone IV (100 mg) was subjected to sodium borohydride reduction in the presence of potassium hydroxide under the same conditions as for the lactol, it was entirely converted to the hemiacetal (chromatoplate evidence). It was characterized by conversion to the acetate which was found identical in all its constants with the 3-acetoxy-2-oxa-euphadiene (Vb).

Seco-A-2-nor-1,3-dihydroxy-euphadiene (VIa) *by lithium aluminum hydride reduction of lactone* IV

A solution of the lactone IV (300 mg) in dry tetrahydrofuran (25 ml) was slowly added to a suspension of lithium aluminum hydride (100 mg) in tetrahydrofuran (50 ml). The mixture was heated to reflux during 3 hr and then decomposed by adding ethyl acetate and a saturated aqueous solution of sodium sulfate. Inorganic salts were removed by filtration, washed with chloroform, and the combined filtrates, dried (Na_2SO_4), were evaporated to dryness. The residue (250 mg) failed to crystallize but was homogeneous on chromatoplate, ν_{max} 3500 and 1043 cm^{-1}.

The *diacetate of* VIa (VIb) was prepared by treating with acetic anhydride and pyridine overnight.

The usual work-up yielded (250 mg) a crude product which was chromatographed on acid washed alumina (Merck). Upon elution with hexane benzene (1:1) the pure diacetate emerged from the column; it could not be induced to crystallize though homogeneous on chromatoplate, $[\alpha]_D$ +27·4° (*c*, 3·0), ν_{max} 1724 and 1242 cm^{-1}.

3-Hydroxy-euphene-2-one (VIIa)

The diosphenol IIa purified by 3 crystallizations (500 mg) dissolved in absolute ethanol (100 ml) was hydrogenated over 10% palladium on charcoal till absorption ceased (2 moles). The filtered solution was concentrated *in vacuo* yielding a foam, ν_{max} 1712 cm^{-1}, negative $FeCl_3$ test, which failed to crystallize, though homogeneous on chromatoplate.

3-Acetoxy-euphene-2-one (VIIb)

(a) *By acetylation of* VIIa. The hydroxy ketone VIIa was acetylated overnight with acetic anhydride and pyridine. The reaction mixture was poured on ice, filtered, washed and dried thoroughly. The crude acetate crystallized from ethanol yielding (200 mg) leaflets, m.p. 160–161°; ν_{max} 1742, 1730 and 1250 cm^{-1}, $[\alpha]_D$ +83·1 (*c*, 0·95). (Found: C, 79·23; H, 10·74. $C_{32}H_{52}O_3$ requires: C, 79·28; H, 10·81%).

(b) *By catalytic hydrogenation of the diosphenol acetate* (IIb). Diosphenol acetate (100 mg) in absolute ethanol (30 ml) was hydrogenated (2 moles) over 10% palladium on charcoal. The filtered solution was distilled *in vacuo* and the residue crystallized from ethanol. The acetoxy ketone (20 mg) was found identical in all respects to the substance obtained by the acetylation procedure in the previous experiment.

2α-Acetoxy-euphene-3-one VIIIb

Pure euphadiene-3-one (I; 1 g) in absolute ethanol (150 ml) was hydrogenated over 10% palladium on charcoal, till absorption ceased (1 mole). The filtered solution was distilled *in vacuo* to a glassy residue of euphene-3-one which was used as such. The substance was dissolved in benzene (200 ml) and part of the solvent distilled to remove traces of water, then cooled and with stirring and under nitrogen, lead tetraacetate (1·3 g containing ~10% acetic acid) was added, followed by boron trifluoride etherate (4 ml). The resulting solution was continuously stirred for 1 hr until negative to iodine-starch paper. The solution was then poured on ice and the organic layer was washed with aqueous sodium bicarbonate and water, then dried (Na_2SO_4). Upon concentration *in vacuo* a brownish glassy residue was obtained which in the I.R. indicated the presence of an acetate group which was introduced. The U.V. spectrum showed low intensity bands at 232, 240, 247 mμ, which are attributed to the formation of a small quantity of a heteroannular diene in rings B,C. The crude product was chromatographed on 25 g of deactivated alumina (acid washed alumina Merck, was deactivated with 5% of a 10% solution of acetic acid). Hexane eluted a mixture of unreacted material, together with some 7,9(11)euphadiene-3-one, followed by the acetoxy ketone VIIIb. The column was subsequently developed with hexane benzene (4:1) which eluted the bulk of the acetoxy ketone. The homogeneous fractions identified on chromatoplate were combined (500 mg) and crystallized from ethanol, m.p. 81–82°, $[\alpha]_D$ +73·9° (*c*, 1·61), ν_{max} 1742, 1730 and 1250 cm^{-1}. (Found: C, 79·20; H, 10·82. $C_{32}H_{52}O_3$ requires: C, 79·28; H, 10·81%).

2-Hydroxy-euphene-3-one (VIIIa)

The acetoxy ketone VIIIb (100 mg) in ethanol solution (10 ml) was boiled for 3 hr under nitrogen with 10 ml of an ethanolic solution of HCl (1 ml conc HCl + 9 ml EtOH). The reaction mixture was diluted with ice water, and extracted with benzene; the benzene extract was washed with aqueous sodium bicarbonate and water, dried (Na_2SO_4) and distilled *in vacuo*. The crude product (80 mg) was chromatographed through deactivated alumina; hexane benzene (4:1) eluted a crystalline product (60 mg) which was homogeneous on chromatoplate; recrystallized from ethanol, m.p. 131–133°, ν_{max} 1718 cm^{-1}.

Isomerization of the acetoxy ketone VIIIb to VIIb

The acetoxy VIIIb (100 mg) in benzene solution was adsorbed on a column packed with basic alumina (Alcoa F20) and left overnight. Elution with benzene yielded the isomeric acetoxy ketone

VIIb which was crystallized from ethanol; found identical in all respects with a sample of the acetoxy ketone VIIb described above. Similar attempts to induce isomerization using acid washed alumina and Florisil failed, the starting material being recovered unchanged.

Isolation of seco-euphene-2,3-dicarboxylic acid anhydride (IX)

During autoxidation of I in the presence of potassium t-butoxide, small quantities of an anhydride were formed with the main reaction product IIa. The anhydride was obtained from the reaction mixture following the hydrogenation procedure (IIa → VIIa) when it crystallized upon concentration of the alcoholic solution. It was collected and recrystallized from ether ethanol and was identified as the anhydride IX, m.p. 192–193°, ν_{max}^{KBr} 1802 and 1759; 1034 and 1014 cm^{-1}. (Found: C, 78·62; H, 10·63. $C_{30}H_{48}O_3$ requires: C, 78·89; H, 10·59%).

Synthesis of anhydride IX

(a) Euphene-3-one (270 mg) in dry t-butanol (10 ml) was added to a saturated solution of potassium t-butoxide in t-butanol (10 ml) and shaken in oxygen till one equivalent of oxygen was absorbed. After the usual work up, the crude 2-hydroxy-1,8-euphadiene-3-one (dihydro IIa) was obtained, ν_{max} 1672 and 1653 cm^{-1}, positive $FeCl_3$, exhibiting two spots on chromatoplate (refer IIa above).

(b) To the above diosphenol in methanol (25 ml), a solution of potassium hydroxyde (2 g) in (20 ml) methanol–water (3:1) was added. To the cooled and stirred solution, 30% hydrogen peroxide (5 ml) was added dropwise at 2–3°. The reaction mixture was then stirred for 2 hr at room temp, then poured on ice, acidified with dil. HCl and extracted with ether. The ether solution was extracted with cold dil. KOH and the aqueous alkaline extract acidified reextracted with ether, the ethereal solution was dried (Na_2SO_4) and distilled *in vacuo* yielding a product (200 mg) with an I.R. spectrum characteristic for an acid: ν_{max} 1704 (broad) cm^{-1}.

(c) The above crude acid (200 mg) was heated for 5 hr on a stream bath with acetic anhydride (5 ml). The reaction mixture was poured on ice, then extracted with benzene, the organic solution was washed with water, dried (Na_2SO_4) and evaporated *in vacuo*. The residue crystallized from ether–ethanol yielding an anhydride, identical in all respects with the product IX obtained as described above.

Acknowledgements—We should like to thank Mrs. R. Tugenhaft for efficient technical assistance and the microanalytical laboratory of our Institute for microanalytical determinations.