DESERT PLANT CONSTITUENTS

II.[1] OCOTILLOL: AN INTERMEDIATE IN THE OXIDATION OF HYDROXY ISOÖCTENYL SIDE CHAINS[1]

E. W. WARNHOFF[2] AND C. M. M. HALLS
Department of Chemistry, University of Western Ontario, London, Ontario
Received July 20, 1965

ABSTRACT

Ocotillol, $C_{30}H_{52}O_3$, a triterpene isolated from *Fouquieria splendens* Engelm., has been found to have structure I containing a new side chain modification. Confirmation was provided by a partial synthesis from dammarenediol-II monoacetate VII*a*. Ocotillol has been found to be an intermediate in one path for the chromic acid oxidation of the hydroxy isoöctenyl side chain XVI, a common feature of several triterpenes, to the tris nor γ-lactone XIX.

In parts of the Sonoran Desert of southwestern North America the most conspicuous form of vegetation is the ocotillo (2).[3] This singular plant (*Fouquieria splendens* Engelm.) (Fig. 1) belongs to a small family (Fouquieriaceae) which has not previously been investigated chemically.[4] Its long branches are covered with a resinous bark and formidable spines. During the wet season the branches also bear leaves (Fig. 1*a*, 1*b*) which drop off at the onset of dry periods (Fig. 1*c*). However, even after the leaves have fallen, the plant is apparently able to synthesize food, for underneath the tough laminated bark there is a chlorophyll layer. Light which reaches this layer could only do so by passing through the translucent bark. Any organic compounds in this resinous bark have thus been subjected to intense desert sunlight for many years, and it would be suprising if some photochemical transformation products had not been formed during this long irradiation period. With the hope of finding such products, a chemical investigation of the plant was initiated.

Isolation and Determination of Structure

Continuous ether extraction of ocotillo bark gave 10% of clear amber resin which was a mixture of 10 or more compounds. Attempts to separate this mixture by alumina chromatography afforded small amounts of two crystalline compounds, m.p. 260.5° and m.p. 200°. Greater success was attained when the resin was first saponified with methanolic potassium hydroxide. When the base-insoluble saponification fraction (60% of the resin weight) was chromatographed on alumina, thin-layer chromatography (t.l.c.) indicated the presence of three major components. One of these (16% of the unsaponified resin) could be purified by recrystallization to give a new compound, ocotillol, $C_{30}H_{52}O_3$, m.p. 198–200°, $[\alpha]_D$ +28°, which was identical with the compound of m.p. 200° that was obtained from the unsaponified resin. However, ocotillol was more conveniently isolated by direct crystallization of its high-melting monoacetate from the acetylation product of the base-insoluble saponification fraction. The infrared spectrum of ocotillol showed the presence of hydroxyl absorption at 3 622 cm^{-1} (secondary OH) and 3 580 cm^{-1} (intramolecularly hydrogen-bonded OH) (5), but no carbonyl peaks. The ultraviolet spectrum

[1]*Part I is considered to be reference 1 in which a preliminary account of some of this work appeared. Presented in part at the 47th Annual Conference of the Chemical Institute of Canada, Kingston, June 1964.*
[2]*Fellow of the Alfred P. Sloan Foundation.*
[3]*Spanish diminutive derivative of the Nahuatl word ocotl, a certain species of pine tree.*
[4]*The results of two earlier general examinations are given in references 3 and 4.*

revealed a complete absence of double bonds, an uncommon feature which temporarily encouraged thoughts of a photochemically transformed triterpene. In agreement, the nuclear magnetic resonance (n.m.r.) spectrum had no vinyl hydrogen peaks, but did have two multiplets (1H each) at δ 3.73 and 3.18 p.p.m. from protons bonded to carbon atoms bearing oxygen functions. Of the eight methyl groups visible in the spectrum, three were at appreciably lower field (δ 1.12–1.21 p.p.m.) than the others. The only structural feature present that could be capable of such deshielding action was oxygen substitution on the carbon atoms bearing these methyl groups.

Reaction with acetic anhydride – pyridine at room temperature gave as the only product a monoacetate, $C_{32}H_{54}O_4$, m.p. 260–261.5°, which proved to be identical with the second crystalline compound from chromatography of the unsaponified resin. The infrared spectrum had an ester carbonyl peak at 1 734 cm^{-1} and only one hydroxyl peak at 3 580 cm^{-1} (intramolecularly hydrogen-bonded OH) (5). An acetate methyl peak appeared at δ 2.03 p.p.m. in the n.m.r. spectrum, and the H—C—O multiplet formerly at δ 3.18 p.p.m. was now shifted to δ 4.50 p.p.m., as expected of the hydrogen on a carbon atom bearing a secondary hydroxyl group (6). More vigorous reaction of the monoacetate with acetic anhydride – pyridine at reflux furnished a diacetate, $C_{34}H_{56}O_5$, which had no hydroxyl infrared absorption. Its n.m.r. spectrum had two acetate methyl peaks at δ 1.96 and 2.03 p.p.m. However, the H—C—O multiplet at δ 3.73 p.p.m. in ocotillol and ocotillol monoacetate had moved only to δ 3.95 p.p.m. Saponification of both acetates regenerated ocotillol. From the facts just presented, ocotillol must be a saturated tetracarbocyclic triterpene with a secondary hydroxyl group, an intramolecularly hydrogen-bonded tertiary hydroxyl group, and an oxide ring with a single hydrogen atom on an α-carbon.[5] The hydroxyl groups were not vicinal since ocotillol was unaffected by sodium metaperiodate.

Oxidation of ocotillol by chromium trioxide in acetic acid at room temperature produced acetone, isolated as the 2,4-dinitrophenylhydrazone, and a keto γ-lactone, $C_{27}H_{42}O_3$, m.p. 182.5–184.5°, $[\alpha]_D$ +71°, as the only products. In like manner ocotillol monoacetate was rapidly oxidized at room temperature with the consumption of 1.37 moles of chromium trioxide per mole of acetate. The only non-volatile product formed in almost quantitative yield was an acetate γ-lactone, $C_{29}H_{46}O_4$. These facile oxidations with loss of three carbon atoms were reminiscent of the oxidation of the dammarenediols VII*b* (7), dipterocarpol VI (8), folientriol (9), and methyl dammarenolate (10). In fact the physical constants for the keto lactone were the same as those reported, m.p. 183–185°, $[\alpha]_D$ +69° to +71°, by three different groups (7, 8, 11) for the keto lactone IV from dammarenediol-II (VII*b*). The methyl peaks in the n.m.r. spectra of the keto γ-lactone and the acetate γ-lactone also corresponded to those reported (12) for IV and V*a*. A direct comparison of the two pairs of compounds revealed them to be identical, thus establishing the structure of the fused ring system of ocotillol. The oxide ring and the other hydroxyl group of the molecule were therefore restricted to the side chain.

Of the four conceivable side chain formulations A, B, C, and D with oxygen at some or all of carbon atoms C-20, C-24, and C-25 as required by the chromic acid oxidation products, three of these can be eliminated. While there are reasonable mechanisms by

[5]*A triol formula, $C_{30}H_{54}O_3$, for ocotillol is not excluded by the analytical data, but it is excluded by the absence of hydroxyl absorption in the infrared spectrum of the diacetate as well as by the amount of chromium VI consumed on oxidation of the monoacetate to the acetate lactone; a triol would have required 2.00 moles of chromium trioxide per mole of monoacetate as compared with the 1.37 moles actually found. After this work was concluded, the mass spectrum of ocotillol was taken by Dr. G. Snatzke in connection with his work on gratiogenin. The molecular ion at m/e 460 confirmed the molecular formula.*

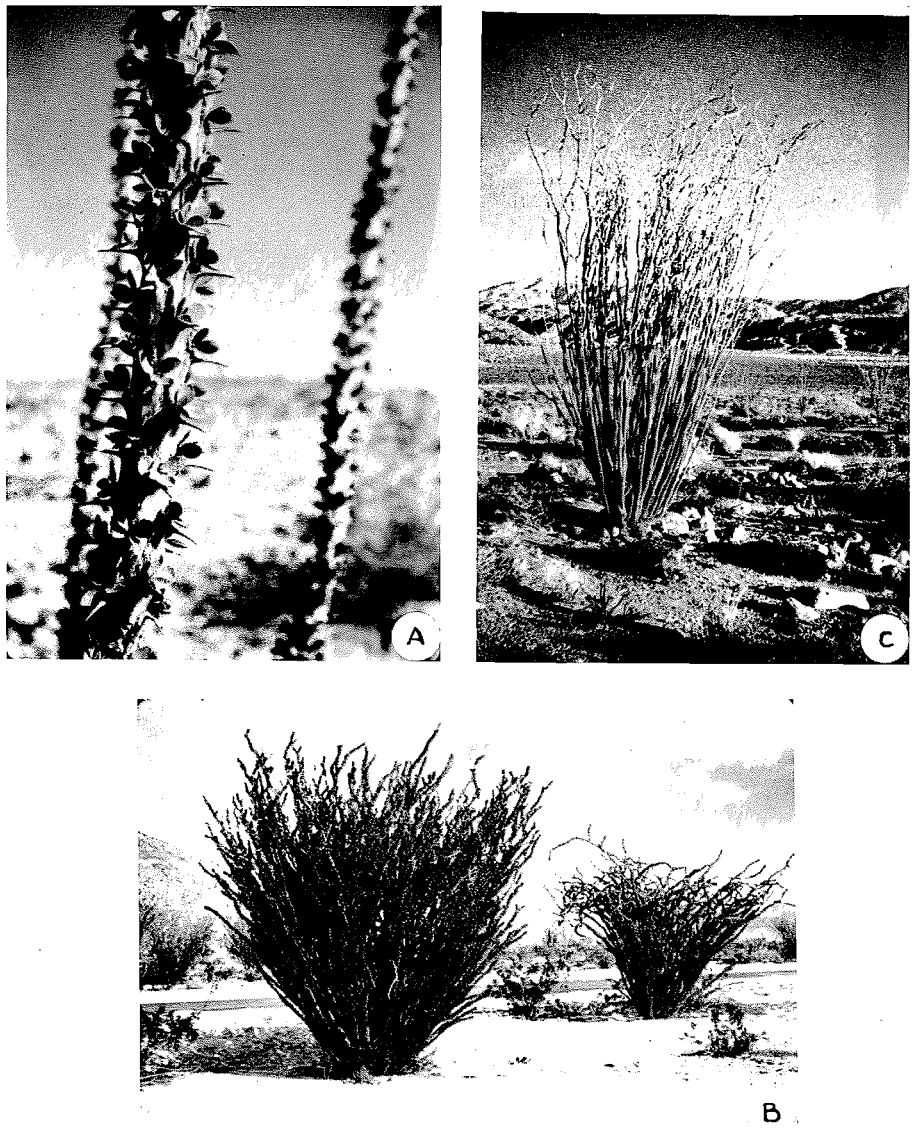
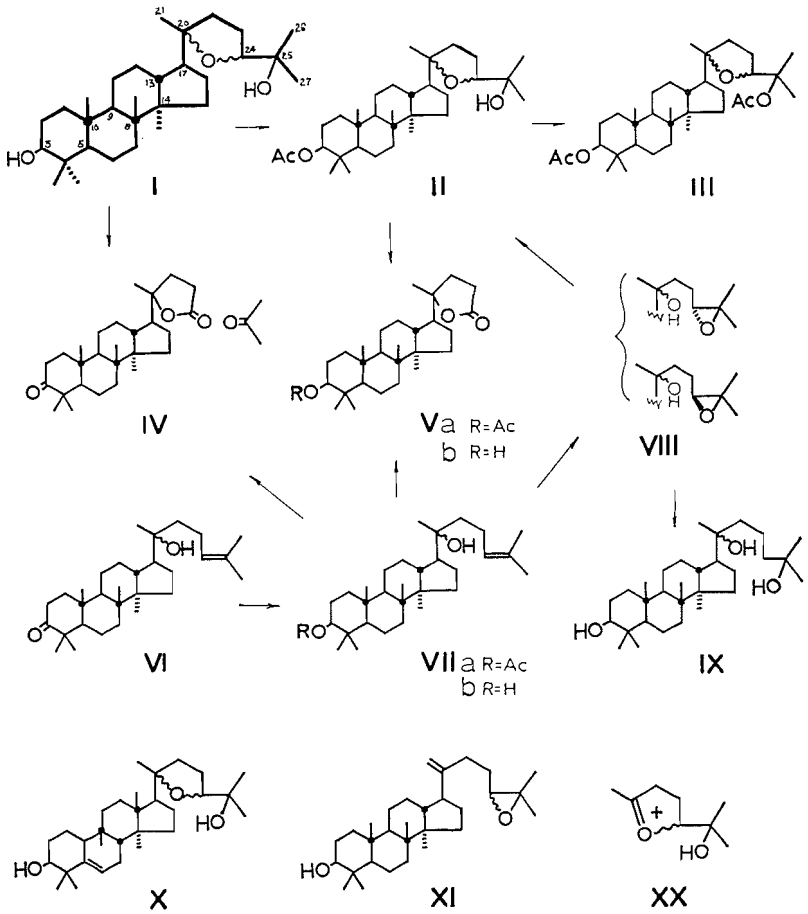
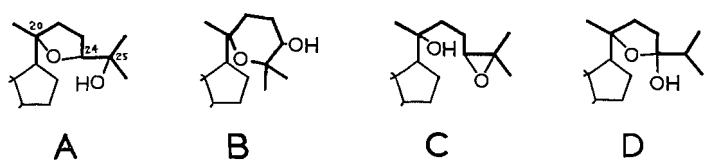


FIG. 1. Ocotillo (*Fouquieria splendens* Engelm.): (*a*) close up of branch, (*b*) in leaf, (*c*) in leafless dry stage.



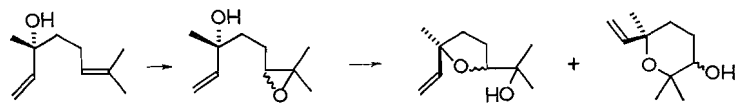
which each could be oxidized in acid solution to a γ-lactone and acetone with the consumption of 1.33 moles of chromium^{VI} per mole of compound, only A is consistent with *all* of the following observations: ocotillol (*a*) forms only a monoacetate at room temperature, (*b*) has three CH_3—C—O and two H—C—O groups, (*c*) is unchanged by anhydrous hydrogen chloride at room temperature, (*d*) is not affected by boiling methanolic potassium hydroxide, (*e*) is recovered from reflux with lithium aluminium hydride in ether or dioxane, and (*f*) is not oxidized by the chromium trioxide – pyridine reagent after monoacetylation. If ocotillol had a tetrahydropyran side chain B, (i) it would have formed the diacetate at room temperature, (ii) *both* H—C—O groups would have been strongly shifted to lower field in the n.m.r. spectrum of the diacetate, and (iii) ocotillol acetate would have been oxidized to a keto acetate by the chromic acid – pyridine reagent since the closely related linalool derivative XV is oxidized to a ketone by this reagent (13, 14).



Therefore, ocotillol has the structure I, its monoacetate formula II, and the diacetate formula III. When this structure was originally proposed (1), the hydroxy tetrahydrofuran appendage was a new terpenoid side chain modification. At about the same time it was recognized in gratiogenin X (15).[6]

Partial Synthesis

The structure I banished consideration of a photochemical origin for ocotillol and suggested that it was probably formed in the plant from a dammarenediol derivative by the biogenetic equivalent of 1,2-epoxidation at C_{24}=C_{25} with subsequent oxide opening at C-24 by the C-20 hydroxyl group. This possibility was later rendered more likely by the discovery in Thailand of a closely related naturally occurring C_{24}—C_{25} oxide, aglaiol XI (16). Although oxide ring opening might occur at C-25, particularly in acid medium, five-membered rings are usually formed more rapidly than six-membered rings. Moreover, there is an excellent analogy for this course of events in the careful work on the so-called linalool oxide (13). Oxidation of R(—) linalool XII with one equivalent of monoperphthalic acid gives as the *primary* product a pair of very sensitive diastereoisomeric 1,2-epoxides XIII and not a mixture of two pairs of diastereoisomeric 1,4- and 1,5-oxides XIV and XV as previously claimed (17). The 1,2-epoxides XIII are very easily converted by acid or heat into a mixture of XIV and XV in the ratio of about 9:1, respectively, the five-membered ring being formed more rapidly even in acidic medium. Consequently, a partial synthesis of ocotillol monoacetate from dammarenediol-II monoacetate VII*a* was undertaken to confirm the structure I.



XII XIII XIV XV

Oxidation of VII*a* under the conditions that produced the true linalool oxide XIII did not permit isolation of the C_{24}—C_{25} epoxides VIII, which are apparently even more easily opened than XIII. Attempts to prepare VIII at lower temperatures or in solution buffered with triethylamine oxide were also unsuccessful. The presence of some 1,2-epoxide could be detected by t.l.c. and was further corroborated by dividing one oxidation product into two fractions, one of which was heated to 100° and the other reduced with lithium aluminium hydride. The thin-layer spot presumed to be epoxides VIII had disappeared in the heated portion with intensification of the spot corresponding to ocotillol monoacetate II, whereas the hydride reduction product contained a new component much more polar than dammarenediol-II (VII*b*) or ocotillol I, which could reasonably be only the triol IX. However, if the epoxidation of VII*a* was allowed to go to completion and worked up as usual, the product gave a single spot corresponding to II on t.l.c. Ocotillol monoacetate was crystallized directly from this product in 35% yield and shown to be identical with the natural material.

The optical rotation and n.m.r. spectrum of the residue from the filtrate betrayed the presence of a second component as well as of II in about equal amounts. From the n.m.r. spectrum and the single t.l.c. spot, the second compound is most probably the C-24 epimer of ocotillol monoacetate, but attempts to crystallize or separate it by chromatography were unsuccessful.

[6]*Dr. Snatzke has informed us that both gratiogenin and ocotillol have as the base peak in their mass spectra the ion m/e 143,* $C_8H_{15}O_2$, *from cleavage of the side chain to XX.*

Stereochemistry

The absolute configurations on the fused ring system of ocotillol at C-3, C-5, C-8, C-9, C-10, and C-14 are fixed by relation to the dammarane skeleton (8, 18), which has in turn been related (18) to *iso*euphol and *iso*tirucallol (19). The β-orientation of the C-13 hydrogen atom follows from the demonstration that the C-13 configuration of the dammarane system is the less stable one. Dammarane derivatives with a carbonyl group at C-17 (20) or at C-12 (21) are epimerized almost completely to the 13-*iso* compounds. The less stable C-13 configuration is β (rings C and D *trans* fused), and molecular rotation differences and conformational analysis support this assignment (20). On the other hand, C-17 has the more stable β-arrangement as witnessed by the stability to base of several C-20 keto derivatives (9, 18, 20). In agreement the coupling constant (10 c.p.s.) between the C-13 and C-17 protons in derivatives with a C-12 keto group is that expected for a *trans* relationship with a dihedral angle of ca. 150° (12). Of the two asymmetric centers in the side chain, the β-configuration of the C-20 hydroxyl has been assigned (21) to folientriol, folientetrol, and the II-series of dammarane compounds, but the grounds on which the assignment was made are erroneous.[7] Thus the stereochemistry at C-20 and C-24 is as yet unknown.

Hydroxy Isoöctenyl Side Chain Oxidation

The quantitative oxidation of the ocotillol side chain to a γ-lactone is merely a special case of the general oxidation of mono ethers of 1,2-diols which Chang and Westheimer (22) have studied with pinacol monomethyl ether. The rapidity of the oxidation suggested that the ocotillol side chain might be an intermediate in the earlier mentioned chromic acid oxidation of the hydroxy *iso*öctenyl side chain XVI of various triterpenes to tris nor γ-lactones XIX. This possibility was tested by oxidation of dammarenediol-II monoacetate (VII*a*) with just the amount of chromium trioxide stoichiometrically required for oxidation to II. From the reaction mixture there was isolated, in addition to lactone V*a* and recovered VII*a*, 6–13% of ocotillol monoacetate II. In view of this result, ocotillol must be an intermediate in one path for the oxidation of VII*a* to V*a*. The hydroxy *iso*öctenyl side chain XVI could produce the hydroxy tetrahydrofuran either by oxidation first to the 1,2-epoxides VIII followed by the rapid oxide opening previously noted, or by oxidation of the double bond to some such cationic intermediate as XVII which is then attacked at C-24 by the C-20 hydroxyl group (XVII → XVIII). Alternatively, the oxidation of the double bond might be an intramolecular process involving a chromate ester of the tertiary C-20 hydroxyl group. The details of this side chain oxidation are being examined further.

EXPERIMENTAL

General

Melting points: Reichert microscope hot stage, corrected.

Optical rotations: Schmidt–Haensch polarimeter, chloroform solutions, 1-dm tube.

Infrared spectra: Beckman IR-5 or IR-7 spectrometer.

Ultraviolet spectra: Bausch and Lomb Spectronic 505 or a Beckman DK spectrometer.

Nuclear magnetic resonance spectra: Varian HR-60 or A-60 spectrometer, 10–20% (g/ml) concentration in deuterochloroform with tetramethylsilane (=O) as internal reference. Integrations by the cut-and-weigh method.

Chromatography: Woelm neutral alumina graded according to the Brockman scale.

Thin-layer chromatography: Camag Kieselgel silica gel with calcium sulphate binder, methanol:chloroform (1.5:98.5) for development, sulphuric acid (33%) for charring. Reported R_f values are taken from one plate on which all compounds were present.

Microanalyses: Dr. A. Bernhardt and associates, Mülheim, Germany.

Petroleum ether refers to the fraction boiling at 60–80°.

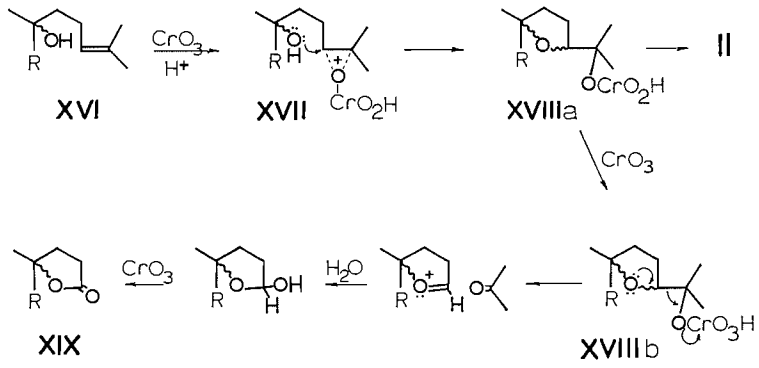
[7]*The assignment was based on a molecular rotation difference between a 20-hydroxydammarane (C-13α?) of unknown C-20 configuration and a dammarane also of unknown C-20 configuration, which had been crystallized from a mixture of dammarane isomers.*

TABLE I

Nuclear magnetic resonance absorption of ocotillol derivatives*

Compound		4α Me	4β Me	10β Me	8β Me	14α Me	21 Me	25 *gem* diMe	3α H	24 H	3β MeCOO	25 MeCOO
Ocotillol	I	0.78	0.85	0.97	0.97	0.87	1.21	1.12	3.18	3.73	—	—
Ocotillol monoacetate	II	0.87	0.87	0.87	0.96	0.87	1.21	1.12	4.50 dd	3.73 dd	2.03	—
Ocotillol diacetate	III	0.85–0.87	0.85–0.87	0.85–0.87	0.96	0.85–0.87	1.11	1.45	4.49 dd	3.95 dd	2.03	1.96
Keto lactone	IV†	0.95	1.04	1.08	1.02	0.92	1.38	—	—	—	—	—
Acetate lactone	Va†	0.85–0.87	0.85–0.87	0.85–0.87	0.96	0.85–0.87	1.37	—	4.49 dd	—	2.04	—

*Chemical shifts are given in p.p.m. from tetramethylsilane (=0) for deuterochloroform solutions.
†The values for these compounds are the same (±0.01 p.p.m.) as those in ref. 12.



Isolation of Ocotillo Resin

Ocotillo bark was collected in Borrego Valley, California, in January, 1962. The loose sandy soil of the region and the shallow root system of the plant make the ocotillo vulnerable to toppling by wind or rain. Since the bark of the fallen and partially dried plants was more easily stripped than that of a freshly cut branch of a living plant, most of the bark was gathered from fallen specimens. The highly resinous bark could not be ground in a Wiley or a hand mill, and instead was cut into small bits with a pair of shears. These pieces, in batches of 20–40 g, were extracted exhaustively with ether in a Soxhlet apparatus. The yield of ether-soluble material per batch varied from 10% to 15% of the weight of the bark. The resin is concentrated in the inner bark, because extraction of the yellow translucent laminated inner bark after separation from the darker outer bark gave 15% of ether-soluble resin, whereas extraction of the whole bark with ether gave only 10–11% of ether-soluble resin. From 6.15 kg of whole bark a total of 618 g (10%) of clear amber resin was obtained. Further extraction of ~~the root~~ bark with methanol gave a small amount of a dark gummy material which was not investigated further. The resin had λ_{max} 269 mμ ($E_{1cm}^{1\%}$ 34), 329 mμ ($E_{1cm}^{1\%}$ 30); $\nu_{max}^{CHCl_3}$ 3 600 and 3 400 cm^{-1} (OH), and 1 725 cm^{-1} (broad, C=O).

The absence of alkaloids in the resin was demonstrated by extraction of an ethyl acetate – chloroform solution of it with dilute hydrochloric acid. The aqueous acid solution gave no precipitate with silicotungstic acid. Basification with sodium hydroxide and extraction with chloroform gave only a trace of residue on evaporation of the dried chloroform solution.

Isolation of Ocotillol Acetate II from Unsaponified Resin (with Mr. J. F. Sollors)

A sample (889 mg) of the unsaponified resin was chromatographed on 30 g of activity I Merck alumina. Only the fractions (58 mg, 6.5%) eluted with ether:benzene (1:3 and 1:1) could be induced to crystallize. These were recrystallized five times from ethyl acetate to give colorless blades, m.p. 260–260.5°, ϵ_{204} 0, ν_{max}^{KBr} 1 724 cm^{-1} (C=O), and 1 250 cm^{-1} (C—O—C). The compound gave a single t.l.c. spot, R_f 0.63, corresponding to ocotillol acetate. The melting point of a mixture with a specimen of the compound from acetylation of ocotillol (see below) (m.p. 257.5–260°) was 257.5–260°, undepressed. There was enough material for only one analysis.

Anal. Calcd. for $C_{32}H_{54}O_4$ (502.75): C, 76.44; H, 10.83. Found: C, 74.99; H, 11.30. Mol. wt. (osmometer, chloroform solution), 513, 517.

A 10-mg sample of the compound was treated under reflux overnight with methanolic potassium hydroxide. Dilution of the reaction mixture with water precipitated a white solid which was recrystallized from 95% ethanol to yield colorless prisms, m.p. 199° (m.p. of ocotillol 198–200°). The infrared spectrum no longer showed carbonyl absorption.

From chromatography of 2.33 g of a different lot of resin there was isolated a few milligrams of I, m.p. 197–200°.

Isolation of Ocotillol I from Saponified Resin

A solution of 10.46 g of ocotillo resin in 350 ml of methanol was heated under reflux with 8 g of potassium hydroxide for 5 h. The reaction mixture was diluted with 1 liter of water and extracted with several portions of ether. The combined ethereal solutions were washed with saturated sodium chloride solution, dried over magnesium sulphate, and filtered. Evaporation of solvents left 6.119 g (ca. 60%) of amber-colored neutral saponification product. Examination by thin-layer chromatography indicated the presence of at least seven compounds of which three were present in considerable amount. Attempts to crystallize the saponification product failed.

The neutral fraction in benzene was poured onto a column of 180 g of grade II alumina, and chromatographed. Benzene:ether (75:25) eluted two fractions weighing 928 mg (15% of the neutral fraction), which crystallized spontaneously and were essentially pure (t.l.c.). Two other fractions weighing 843 mg (13% of

the neutral fraction) crystallized on trituration with ether; these contained a small amount of two contaminants. The total yield of almost pure ocotillol was about 28% of the neutral fraction or approximately 16% of the unsaponified resin. Several recrystallizations of the 928-mg fraction from benzene – petroleum ether or ethyl acetate – petroleum ether gave colorless thin prisms of pure ocotillol I, m.p. 198–200°, $[\alpha]_D^{25}$ +28.3° (*c*, 2.42), $[\alpha]_D^{25}$ +25.7° (*c*, 2.29), R_f 0.36, ϵ_{205}^{EtOH} 228, $\nu_{max}^{CCl_4}$ (IR-7) 3 622 cm^{-1} and 3 580 cm^{-1} (OH) (conc. 0.005 *M*).[8] Integration of the n.m.r. spectrum gave a total of 52–54 hydrogen atoms.

Anal. Calcd. for $C_{30}H_{52}O_3$ (460.72): C, 78.20; H, 11.38; O, 10.42. Found: C, 78.17; H, 11.02; O, 10.90. Mol. wt. (mass spectrum), 460.

From the following experiments ocotillol was recovered almost quantitatively. In each case t.l.c. examination showed I to be the only product.

(*a*) A solution of 19 mg of I and 19 mg of sodium metaperiodate in 3.25 ml of water:dioxane (5:8) was allowed to stand for 140 min at room temperature.

(*b*) A solution of 10 mg of I in 3 ml of chloroform was saturated with anhydrous hydrogen chloride and allowed to stand overnight at room temperature.

(*c*) A solution of 11.2 mg of I in 8 ml of ether was treated by reflux with 87 mg of lithium aluminium hydride for 3.5 h.

(*d*) A solution of 11.4 mg of II in 20 ml of dioxane was treated by reflux with 191 mg of lithium aluminium hydride for 8 h (yield: 6 mg of I).

Ocotillol Monoacetate II

From Pure Ocotillol

A solution of 286 mg (0.62 mmole) of ocotillol in 3 ml of dry pyridine and 0.2 ml of acetic anhydride was allowed to stand for 24 h at room temperature. The reaction mixture was diluted with water and extracted with chloroform and ether. The combined chloroform–ether extracts were washed successively with dilute sulphuric acid, water, sodium bicarbonate solution, and water. The dried (sodium sulphate) and filtered solution was evaporated, to leave 284 mg (91%) of crystalline residue, which t.l.c. showed to be almost pure monoacetate. Four recrystallizations from ethyl acetate – petroleum ether or chloroform – petroleum ether gave colorless prisms of ocotillol monoacetate II, m.p. 260–261.5°, $[\alpha]_D^{25}$ +41° (*c*, 1.13), $[\alpha]_D^{22}$ +39.5° (*c*, 2.52) and +40.5° (*c*, 2.55), R_f 0.63; $\nu_{max}^{CCl_4}$ (IR-7) 3 580 cm^{-1} (OH), 1 734 cm^{-1} (ester C=O) (conc. 0.005 *M*); $\nu_{max}^{CHCl_3}$ 3 546 cm^{-1} (OH), 1 718 cm^{-1} (ester C=O), and 1 256 cm^{-1} (C—O—C).

Anal. Calcd. for $C_{32}H_{54}O_4$ (502.74): C, 76.44; H, 10.83. Found: C, 76.85; H, 10.99.

From Neutral Saponification Product

A solution of 10 g of the neutral fraction of the resin saponification product in 80 ml of dry pyridine and 8 ml of acetic anhydride was allowed to stand for 24 h at room temperature. The reaction mixture was worked up as in the preparation of the monoacetate from pure ocotillol, except that 10% potassium hydroxide solution was used in place of sodium bicarbonate solution. The acetylated product (9.7 g) was dissolved in ethyl acetate – petroleum ether, concentrated to about 200 ml, and seeded with pure II. After several days of slow crystallization first at room temperature and then at 3°, filtration gave 953 mg (ca. 8.7%) of colorless thin prisms (almost pure II), m.p. 222–243°, in three crops. Two recrystallizations from ethyl acetate – petroleum ether gave pure ocotillol monoacetate identical with that prepared as above from pure ocotillol.

Saponification

A solution of 71 mg of ocotillol monoacetate II and 0.4 g of potassium hydroxide in 20 ml of methanol was heated under reflux for 27.5 h. Dilution with water and extraction with ether gave 51 mg (78%) of ocotillol, m.p. 193.5–197°, which gave a single t.l.c. spot. One recrystallization from benzene – petroleum ether raised the melting point to 198–198.5°, undepressed on admixture with authentic ocotillol. The infrared spectrum was identical with that of ocotillol.

Ocotillol Diacetate III

Preparation

Ocotillol monoacetate (252 mg, 0.50 mmole) was treated by reflux for 48 h in 5 ml of pyridine with 0.5 ml of acetic anhydride. The reaction mixture was diluted with water, extracted with ether, and worked up as for the monoacetylation of pure ocotillol. The crude dark brown product (278 mg) was chromatographed on 10 g of activity II alumina. Benzene – petroleum ether (50:50) eluted 193 mg (70%) of colorless crystals which gave a single t.l.c. spot, R_f 0.74. Three recrystallizations from methanol – methylene chloride gave colorless flakes of ocotillol diacetate III, m.p. 199–202°, $[\alpha]_D^{21}$ +38° (*c*, 2.60), $\nu_{max}^{CCl_4}$ 1 736 (ester C=O) and 1 250 cm^{-1} (C—O—C).

Anal. Calcd. for $C_{34}H_{56}O_5$ (544.79): C, 74.95; H, 10.36. Found: C, 75.23; H, 10.49.

An attempted acetylation in pyridine – acetic anhydride at 90° for 24 h (conditions used for diacetylation of gratiogenin X (15)) gave back monoacetate containing only a small amount of diacetate.

Saponification

A 22-mg sample of the diacetate was treated by reflux for 48 h with 88 mg of potassium hydroxide in 15 ml of methanol. Dilution with water and extraction with ether gave 19 mg of a solid, which was recrystallized

[8]*For comparison, a 0.005 M solution of 3β-cholestanol had* $\nu_{max}^{CCl_4}$ *(IR-7) 3 620* cm^{-1}.

from benzene – petroleum ether to yield 12.4 mg of ocotillol, m.p. 197–198°, undepressed on admixture with an authentic specimen.

Oxidation of Ocotillol Monoacetate II

Chromic Acid – Acetic Acid

A solution of 303 mg (0.61 mmole) of II in 41 ml of glacial acetic acid was added to 91 mg (0.91 mmole) of chromium trioxide in 5 drops of water and 2 ml of glacial acetic acid. The reaction mixture was almost green after 1 h. After 24 h the emerald-green solution was treated with potassium iodide. Titration of the iodine liberated by excess chromium trioxide required 2.39 ml of 0.100 *N* sodium thiosulphate (corresponding to 7.9 mg of chromium trioxide). The 83.1 mg (0.83 mmole of chromium ^{VI} consumed in the oxidation was 102% of the theoretical amount required for oxidation of II to the lactone V*a* and acetone.

The titrated reaction mixture was diluted with water and extracted with ether. The ether extracts were washed with thiosulphate solution, water, sodium bicarbonate solution, saturated sodium chloride solution, and then dried over sodium sulphate. Evaporation of the filtered solution left 347 mg of crystalline product, which retained some solvent and gave a single t.l.c. spot, R_f 0.71. One recrystallization from methanol – methylene chloride gave 211 mg (75%) of prisms of acetate lactone V*a* (3β-hydroxy tris nor dammarane 24:20 ξ_2-olide acetate), m.p. 242–245.5° unchanged on further recrystallization, $[\alpha]_D$ +42° (*c*, 2.24); $\nu_{max}^{CHCl_3}$ 1 761 cm^{-1} (γ-lactone C=O), 1 721 cm^{-1} (ester C=O), and 1 253 cm^{-1} (C—O—C) (lit. (8): m.p. 237–238°, $[\alpha]_D$ +50°). The melting point was undepressed on admixture with V*a* from dipterocarpol (8).

Anal. Calcd. for $C_{29}H_{46}O_4$ (458.66): C, 75.94; H, 10.11. Found: C, 75.66; H, 10.04.

From another reaction in which 150 mg of II was oxidized the yield of crude acetate lactone (one t.l.c. spot) was 136 mg (99%).

A solution of 136 mg of acetate lactone in 30 ml of methanol was saponified by reflux with 564 mg of potassium hydroxide. Acidification with dilute sulphuric acid and extraction with ether gave 123 mg of hydroxy lactone V*b* (single t.l.c. spot), which after four recrystallizations from methanol–water had m.p. 208.5–210°, $[\alpha]_D$ +25° (*c*, 1.42, chloroform containing 5% of 95% ethanol) (lit. (8): m.p. 205–206°, $[\alpha]_D$ +39°).

Chromium Trioxide – Pyridine

To the oxidizing agent prepared from 14.3 mg (0.143 mmole) of chromium trioxide and 0.5 ml of pyridine was added a solution of 100 mg (0.207 mmole) of II in 9 ml of pyridine. After 4.7 h at room temperature the reaction mixture was diluted with 40 ml of methylene chloride and poured onto a column of 3 g of activity III alumina. Further elution with 160 ml of methylene chloride gave a colorless eluate which was washed successively with dilute sulphuric acid, water, sodium bicarbonate solution, water, and saturated sodium chloride solution. The dried solution (sodium sulphate) was concentrated, and left 99 mg (99%) of recovered ocotillol monoacetate, m.p. 250.5–254°, $[\alpha]_D^{25}$ +36° (*c*, 2.50), which gave a single t.l.c. spot. The melting point was undepressed on admixture with the starting material, and the n.m.r. spectrum was identical with that of the starting material.

Oxidation of Ocotillol I

A mixture of 202 mg (0.44 mmole) of I, 98 mg (0.98 mmole) of chromium trioxide, and 7 drops of water in 17 ml of glacial acetic acid was allowed to react at room temperature for 24 h. Then a solution of 11 mg of sodium bisulphite in 50 ml of water was added and the reaction mixture was steam distilled. The distillate (25 ml) was treated with acidic 2,4-dinitrophenylhydrazine solution and the resulting orange precipitate (30 mg, 28%) was collected on a filter. Two recrystallizations from ethanol gave 14 mg of prisms of acetone 2,4-dinitrophenylhydrazone, m.p. 125–126°. On admixture with an authentic sample, m.p. 125–126.5°, the melting point was 124.5–125.5°. The infrared spectra of both samples in chloroform were identical.

The residue from the steam distillation was extracted with three portions of ether. The combined ethereal solutions were washed in sequence with water, sodium bicarbonate solution, water, and saturated sodium chloride solution before being dried over anhydrous sodium sulphate. Evaporation of the ether left 181 mg (99%) of crystalline residue, m.p. 173.5–181.5°, which gave a single t.l.c. spot. Three recrystallizations from absolute ethanol – petroleum ether gave 101 mg of colorless prisms of the keto lactone IV, m.p. 182.5–184.5°, $[\alpha]_D^{25}$ +71° (*c*, 2.51), ϵ_{210}^{EtOH} 55; $\nu_{max}^{CHCl_3}$ 1 757 cm^{-1} (γ-lactone), 1 698 cm^{-1} (6-ring C=O), 1 421 cm^{-1} (CH_2 C=O); $\nu_{max}^{CCl_4}$ 1 775 cm^{-1} (γ-lactone) and 1 706 cm^{-1} (6-ring C=O). On admixture with a sample of the tris nor lactone from dipterocarpol VI (≡tris nor keto lactone from dammarenediol-II), the melting point was undepressed. The infrared spectra in chloroform solution were identical. Both samples had the same R_f 0.67 on t.l.c.

Anal. Calcd. for $C_{27}H_{42}O_3$ (414.61): C, 78.21; H, 10.21; O, 11.58. Found: C, 77.84; H, 10.13; O, 11.76. Mol. wt. (mass spectrum), 414.

A blank oxidation with the same reagents but with the omission of I, followed by distillation and addition of 2,4-dinitrophenylhydrazine gave no precipitate. However, extraction of the treated distillate and chromatography on silica gel did give 12 mg of a mixture of dinitrophenylhydrazones of which the acetone derivative constituted about 6 mg. Since this is much less than the *precipitate* collected in the ocotillol oxidation, acetone is one of the products of oxidation of ocotillol.

Dammarenediol-II Monoacetate VIIa from Dipterocarpol VI

A solution of 727 mg of sodium borohydride in 40 ml of dioxane–water (1:1) was added to a solution of

1.781 g of dipterocarpol VI (≡20-hydroxydammarenone-II), R_f 0.73, m.p. 124–134°, $[\alpha]_D^{25}$ +62° (*c*, 3.47) (lit. (8): m.p. 127° and 135–136°, $[\alpha]_D$ +65°) in 60 ml of dioxane. Work-up gave 1.732 g of crude product, which was recrystallized from methanol. There was obtained 1.482 g (85%) of colorless flakes of dammarenediol-II (VII*b*), m.p. 78.5–82° (lit. (7): m.p. 131–133°), R_f 0.44, which were either solvated or polymorphic.

Acetic anhydride (1.2 ml) was added to a solution of 1.48 g of VII*b* in 12 ml of pyridine, and the reaction mixture was allowed to stand at room temperature for 26 h. Work-up as for the monoacetylation of ocotillol gave 1.571 g of yellow resin. Recrystallization from methanol yielded 1.351 g (83%) of dammarenediol-II monoacetate VII*a*, m.p. 130–134°, $[\alpha]_D^{25}$ +38° (*c*, 2.68) (lit. (7): m.p. 135–137°, $[\alpha]_D$ +37°), R_f 0.73.

*Epoxidation of Dammarenediol-II Monoacetate VII*a

To a solution of 299 mg (0.615 mmole) of VII*a* in 15 ml of ether chilled in a bath of Dry Ice and acetone, 1.5 ml of an ethereal solution of monoperphthalic acid (0.687 mmole) was added. The reaction mixture was stored at −10° for 210 h, then diluted with chloroform and washed successively with sodium bicarbonate solution, water, and saturated sodium chloride solution. Evaporation of the dried ether solution (water bath temperature = 90°) left 263 mg of colorless solid, which was recrystallized from ethyl acetate – petroleum ether. Seeding with a crystal of ocotillol acetate produced 110 mg (35%) of prisms, m.p. 247–248°, $[\alpha]_D^{25}$ +37° (*c*, 2.46). Three more recrystallizations from the same solvent gave 65 mg of pure ocotillol monoacetate (II), m.p. 260–260.5°, $[\alpha]_D^{25}$ +40° (*c*, 2.50), R_f 0.63. The melting point was undepressed on admixture with an authentic sample prepared as described above. The infrared and n.m.r. spectra were identical with those of authentic II.

Anal. Calcd. for $C_{32}H_{54}O_4$ (502.74): C, 76.44; H, 10.83. Found: C, 76.58; H, 10.95.

In an attempt to isolate the C-24 epimer of ocotillol monoacetate, the mother liquors from the first crop of crystals were combined with those from another reaction and chromatographed on activity II alumina. Various fractions which gave a single t.l.c. spot corresponding in R_f to II had optical rotations ranging from $[\alpha]_D$ +31° to $[\alpha]_D$ +34°, significantly lower than II or VII*a*. The n.m.r. spectrum of this material differed from that of ocotillol monoacetate in that there were two short methyl peaks at 1.11 and 1.13 p.p.m. instead of one tall methyl peak at ~1.12 p.p.m. The material was apparently a mixture of II and its C-24 epimer in about equal amounts; however, the C-24 epimer could not be crystallized from the mixture. A calculation based on the optical rotations of pure II and the estimated composition of the mixture gives a value of $[\alpha]_D$ ca. +24° for the C-24 epimer of ocotillol monoacetate.

*Chromic Acid Oxidation of Dammarenediol-II Monoacetate VII*a

Dammarenediol-II monoacetate (249 mg, 0.51 mmole) shown by t.l.c. to be free of I and II in 6 ml of glacial acetic acid containing 10 drops of water was oxidized with 33.5 mg (0.33 mmole, the theoretical amount for oxidation of VII*a* to II) of chromium trioxide at room temperature. After 14 h the reaction mixture was diluted with water and worked up as for the oxidation of ocotillol monoacetate. Thin-layer chromatography of the 237 mg of crude product indicated the presence of the acetate lactone and ocotillol monoacetate as well as unreacted starting material. To remove the lactone the product was treated by reflux in a mixture of 15 ml of methanol, 5 ml of water, and 175 mg of potassium hydroxide for about 4 h. Dilution with water and extraction of the basic solution with chloroform and ether gave 159 mg of neutral material, which was mainly a mixture of I and VII*b*. Acidification of the basic aqueous layer with dilute sulphuric acid and extraction with ether yielded 46 mg (21%) of hydroxy lactone V*b*. Acetylation of this with pyridine – acetic anhydride at room temperature gave 50 mg of acetate lactone V*a* which after two recrystallizations from methylene chloride – methanol had m.p. 241–244.5°, undepressed on admixture with an authentic sample.

The 159 mg of neutral product was dissolved in benzene – petroleum ether and chromatographed on 5 g of grade III alumina. Benzene and benzene:ether (98:2) eluted 55 mg (24%) of dammarenediol-II (VII*b*), which was identified by t.l.c. and conversion to the acetate VII*a*. Benzene:ether (98:2 to 90:10) eluted 58 mg, which t.l.c. showed to be about half II (~13% yield). Five recrystallizations from benzene – petroleum ether gave ocotillol of m.p. 195–196°, undepressed on admixture with authentic I. Acetylation of the recrystallization mother liquors gave 53 mg, which was estimated by t.l.c. to contain ca. 14 mg (~6% yield from VII*a*) of II. Three recrystallizations from ethyl acetate – petroleum ether gave 4 mg of pure II, m.p. 254–255°, undepressed on admixture with an authentic sample.

Dammarenediol-II monoacetate gave little or no ocotillol monoacetate II on oxidation with the chromic acid – pyridine reagent.

ACKNOWLEDGMENTS

The authors would like to thank the National Research Council for support, Professor G. Ourisson for the authentic specimens of IV and V*a*, and Dr. G. Snatzke, Bonn, for the mass spectra and a copy of his manuscript in advance of publication. They are also indebted to Mr. J. F. Sollors for the experiment indicated.

REFERENCES

1. C. M. M. HALLS and E. W. WARNHOFF. Chem. Ind. London, 1986 (1963).
2. L. BENSON and R. A. DARROW. The trees and shrubs of the southwestern deserts. 2nd ed. University of Arizona Press, Tucson, Arizona. 1954. pp. 270–271.
3. H. C. DE S. ABBOTT. Am. J. Pharm. **57**, 81 (1885).
4. E. SCHAER. Arch. Pharm. **236**, 1 (1898).
5. A. R. H. COLE. *In* Elucidation of structures by physical and chemical methods. Part I. *Edited by* K. W. Bentley. Interscience Publishers, New York, N.Y. 1963. pp. 144–151.
6. J. N. SHOOLERY and M. T. ROGERS. J. Am. Chem. Soc. **80**, 5133 (1958).
7. J. S. MILLS and A. E. A. WERNER. J. Chem. Soc. 3132 (1955).
8. P. CRABBÉ, G. OURISSON, and T. TAKAHASHI. Tetrahedron, **3**, 279 (1958).
9. F. G. FISCHER and N. SEILER. Ann. **626**, 185 (1959).
10. D. ARIGONI, D. H. R. BARTON, R. BERNASCONI, C. DJERASSI, J. S. MILLS, and R. E. WOLFF. J. Chem. Soc. 1900 (1960).
11. L. VAN ITALLIE. Arch. Pharm. **250**, 204 (1912).
12. J.-M. LEHN. Bull. Soc. Chim. France, 1832 (1962).
13. D. FELIX, A. MELERA, J. SEIBL, and E. sz. KOVÁTS. Helv. Chim. Acta, **46**, 1513 (1963).
14. E. KLEIN, H. FARNOW, and W. ROJAHN. Tetrahedron Letters, **17**, 1109 (1963). E. KLEIN, H. FARNOW, and W. ROJAHN. Ann. **675**, 73 (1964).
15. R. TSCHESCHE, G. BIERNOTH, and G. SNATZKE. Ann. **674**, 196 (1964).
16. D. SHIENGTHONG, A. VERASARN, P. NANONGGAI-SUWANRATH, and E. W. WARNHOFF. Tetrahedron, **21**, 917 (1965).
17. M. MOUSSERON-CANET, C. LEVALLOIS, and J. WYLDE. Tetrahedron Letters, **17**, 769 (1962).
18. J. S. MILLS. J. Chem. Soc. 2196 (1956).
19. (*a*) D. ARIGONI, R. VITERBO, M. DÜNNENBERGER, O. JEGER, and L. RUZICKA. Helv. Chim. Acta, **37**, 2306 (1954). (*b*) D. ARIGONI, O. JEGER, and L. RUZICHA. Helv. Chim. Acta, **38**, 222 (1955). (*c*) D. H. R. BARTON, J. F. MCGHIE, M. K. PRADHAN, and S. A. KNIGHT. J. Chem. Soc. 876 (1955)
20. J.-F. BIELLMANN, P. CRABBÉ, and G. OURISSON. Tetrahedron, **3**, 303 (1958).
21. F. G. FISCHER and N. SEILER. Ann. **644**, 146 (1961).
22. Y. W. CHANG and F. H. WESTHEIMER. J. Am. Chem. Soc. **82**, 1401 (1960).