Tetrahedron, 1963, Vol. 19, pp. 1285 to 1291. Pergamon Press Ltd. Printed in Northern Ireland

ESTAFIATIN, A NEW SESQUITERPENE LACTONE ISOLATED FROM *ARTEMISIA MEXICANA* (WILLD)[1,2]

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(*Received* 25 *January* 1963)

Abstract—The structure of estafiatin, a constituent of *Artemisia mexicana* W. has been established as a sesquiterpenic epoxylactone of the guaiane series.

Artemisia mexicana (Willd) is a bitter herb common in the central plateau and the northern part of Mexico. It is known popularly as "Estafiate" and extracts of this plant have been used as an antihelminthic. Rio de la Loza reported the occurrence of santonin, a finding which could not be confirmed subsequently. In connection with a study now under way in this Laboratory on the constituents of mexican plants which possess medicinal properties, we became interested in this one.

We were not able to isolate santonin on carefully chromatographing the chloroform extract of *Artemisia mexicana*, it might be pointed out that the plant we processed was collected in November, at the end of its growth cycle and any santonin present could have suffered further transformations. We isolated a large amount of oily lactonic material which resisted crystallization after repeated chromatographic separations.

The present paper is concerned with the isolation and structure proof of a new sesquiterpene lactone which we propose to name estafiatin after the popular name of the plant.

Estafiatin (I) was eluted in the less polar fractions of the chromatogram, it is a crystalline substance $C_{15}H_{18}O_3$, m.p. 104–106°, $[\alpha]_D$ −9·9°. The I.R. spectrum had bands at 1755 and 1668 cm^{-1} (α,β-unsaturated lactone), at 1640 and 903 cm^{-1} (exocyclic methylene group). The U.V. spectrum indicated also the presence of an α,β-unsaturated lactone (λ_{max} 214 mμ; ε, 9850) very probably of the same type as found in helenaline.[3,4]

This is supported by the following evidence. Estafiatin showed only one methyl group in the C-methyl determination. Ozonolysis gave rise to formaldehyde; there was isolated also from this reaction a bisnordiketone, $C_{13}H_{14}O_5$ (II) which exhibited the properties of an enolized α-ketolactone;[5] λ_{max} 236 mμ; ε, 10700, positive ferric chloride test. The I.R. spectrum had bands at 3500 cm^{-1} (hydroxyl group), at 1750 cm^{-1} (α-enolic γ-lactone) and at 1705 cm^{-1} (cycloheptanone). Estafiatin yielded two isometric pyrazolines on treatment with ethereal diazomethane. The N.M.R.

[1] Taken in part from a thesis by F. Sánchez-Viesca, Universidad Nacional Autónoma de México.

[2] An abstract was presented at *Octavo Congreso Latinoamericano de Química* Buenos Aires, September, (1962). Contribution No. 150 from the Instituto de Química de la UNAM.

[3] R. Adams and W. Herz, *J. Amer. Chem. Soc.* **71**, 2346, 2551, 2554 (1949).

[4] G. Büchi and D. Rosenthal, *J. Amer. Chem. Soc.* **78**, 3860 (1956).

[5] W. Herz, H. Watanbe, M. Miyazaki and Y. Kishida. *J. Amer. Chem. Soc.* **84**, 2601 (1962) and references cited therein.

spectrum[6] of estafiatin exhibited in the vinyl proton region two low field doublets (intensity one proton each) centered at 5·48 and 6·18 p.p.m. associated with the unsaturated lactone system (J = 3·3 c.p.s.) and a pair of slightly split signals at 4·86 and 4·95 p.p.m. corresponding to the isolated exocyclic methylene group (J = 2); it showed in the methyl region a displaced sharp signal (singlet, intensity 3 protons) at 1·6 p.p.m. ascribed to a methyl group on a fully substituted carbon atom.

Chemical reduction of estafiatin with amalgamated aluminium, saturated only the exocyclic double bond conjugated with the lactone yielding a dihydroderivative (III). Partial catalytic hydrogenation yielded the same substance in low yield. Its U.V. spectrum had a low field maximum (λ_{max} 205 mμ; ε, 3000) corresponding to a disubstituted C—C double bond.[7] The I.R. spectrum had bands at 1760 cm^{-1} (γ-lactone), at 1640 and 900 cm^{-1} (exocyclic methylene group). The C-methyl determination on dihydroestafiatin (III) indicated the presence of two methyl groups and yielded formaldehyde on ozonolysis. The N.M.R. spectrum displayed in the vinyl proton region a singlet at 4·82 p.p.m. (intensity 2 protons) (the shape of the signal indicates some splitting which is not resolved). In the methyl region exhibited a sharp signal at 1·53 p.p.m. (singlet) and a new doublet at 1·18 p.p.m. corresponding to a methyl group on a secondary carbon atom; which arises from the reduction of the double bond conjugated with the lactone. There was obtained from this hydrogenation in small yield an isomeric dihydroestafiatin. We assign to this derivative structure (IV) in which the nonconjugated exocyclic methylene group was saturated and the double bond conjugated with the lactone was isomerized under the influence of the catalyst since the U.V. spectrum (λ_{max} 218 mμ; ε, 10800) and the I.R. bands at 1740 and 1668 cm^{-1} indicated the presence of the α,β-unsaturated lactone chromophore. This compound was recovered unchanged after treatment with ethereal diazomethane. A similar shift is observed in the hydrogenation of ambrosin[8,9] and parthenin.[5]

Saturation of the two C—C double bonds of estafiatin by hydrogenation in the presence of Adams catalyst, did not occur stereospecifically and a complex mixture was obtained. By repeated chromatography three crystalline tetrahydro stereoisomers could be isolated. They are designated as tetrahydroestafiatins a, b and c. Their I.R. spectra did not show >C=C< bonds.

We next turned our attention to the determination of the carbon skeleton of estafiatin. Pyrolysis of the crude oily mixture of tetrahydroestafiatins with selenium afforded chamazulene which was characterized as the trinitrobenzene adduct.[10] The properties of estafiatin were therefore interpreted on the basis of the guaiane structure. The unconjugated double bond was placed as as exocyclic methylene located at C_{10} since the I.R. band at 1705 cm^{-1} exhibited by the bisnordiketone (II) corresponds to a cycloheptanone. A similar band (at 1700 cm^{-1}) is observed in the I.R. spectrum of dihydronorestafiatone (VI) obtained by ozonolysis of dihydroestafiatin (III). In the methyl region of the N.M.R. spectrum, the ketone (VI) had the sharp signal at 1·6

[6] The N.M.R. spectra were run by Drs. José Luis Mateos and Fernando Walls to whom we are indebted; on a Varian A-60 spectrophotometer, in chloroform solution, using tetramethylsilane as internal standard.

[7] O. H. Wheeler and J. L. Mateos, *J. Org. Chem.* **21**, 1110 (1956).

[8] L. Bernardi and G. Büchi, *Experientia* **13**, 466 (1957).

[9] F. Sorm, M. Suchy and V. Herout, *Coll. Czechoslov. Chem. Comun.* **24**, 1548 (1959).

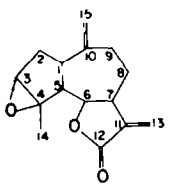
[10] We are grateful to Dr. F. Sorm for sending us samples of chamazulene and artemazulene trinitrobenzene adducts.

p.p.m. (intensity 3 protons) and a doublet at 1·23 p.p.m. established the presence of a $>CH—CH_3$ grouping.

The third oxygen atom is not present in the form of a hydroxyl or carbonyl group, since the I.R. spectrum did not show bands of hydroxyl or other carbonyl group apart from that of the lactone. Estafiatin was recovered unchanged after treatment with acetic anhydride and pyridine; carbonyl reagents did not produce a derivative under usual conditions. We could find evidence that this oxygen atom is present as an epoxide, in the following way. Treatment of estafiatin (I) with a solution of hydrogen chloride in chloroform yielded a chlorohydrin whose infrared spectrum had bands at 3580 cm^{-1} (hydroxyl group), at 1783 cm^{-1} (γ-lactone). The two exocyclic methylene groups are intact as shown by the bands at 902, 1642 and 1668 cm^{-1}. The U.V. spectrum (λ_{max} 214 mμ; ε, 8500) indicates the presence of the α,β-unsaturated lactone. Boron trifluoride etherate smoothly isomerized the epoxide of estafiatin to a ketone (VII) (λ_{max} 211 mμ; ε, 9460). It yielded an orange 2,4-dinitrophenylhydrazone ($\lambda_{max}^{CHCl_3}$ 362–364 mμ; ε, 24100) corresponding to a saturated ketone.[11] The I.R. spectrum of estafiatone (VII) (bands at 1760, 1740, 1668, 1640 and 900 cm^{-1}) afforded information about the relative position of the epoxide in the perhydroazulene skeleton since the new band at 1740 cm^{-1} corresponds to a cyclopentanone. The following data allow us to assign to the epoxide a definite position in the cyclopentane ring. Boron trifluoride isomerization of dihydronorestafiatone (VI) yielded a diketone (VIII) which did not exhibit the properties of a β-diketone (negative ferric chloride test). Alkaline treatment of tetrahydroestafiatin-a (V) causes openings at the epoxide and gives rise to a vicinal glycol (XIIa) (consumption of periodic acid). One of its hydroxyl groups has a tertiary character since only a monoacetate (XIIb) was obtained by acetylation with acetic anhydride and pyridine. This acetate showed an hydroxyl band at 3570 cm^{-1} in the I.R. spectrum. In this way positions $C_1—C_2$, $C_2—C_3$ are excluded. The epoxide could not be attached to the tertiary positions $C_4—C_5$ since the isomerization to a ketone could not occur. There only remains $C_3—C_4$ for the insertion of this function. Comparison of the methyl region in the N.M.R. spectra of estafiatin (I) and estafiatone (VII) fully substantiates this assumption. The displaced sharp signal at 1·6 p.p.m. of estafiatin (*vide supra*) is replaced in estafiatone by a doublet at 1·22 p.p.m. characteristic of a $>CH—CH_3$ grouping. In the vinyl proton region estafiatone exhibited two doublets at 5·53 and 6·19 p.p.m. ($J = 3·1$) ascribed to the protons at C_{13} and two singlets at 4·66 and 4·99 p.p.m. corresponding to the exocyclic methylene group attached at C_{10}. Several derivatives of estafiatone were prepared. Catalytic hydrogenation of estafiatone (VII) only reduces the unconjugated double bond and the resulting dihydroderivative (IX) resisted further hydrogenation. The position of the U.V. maximum (λ_{max} 220 mμ; ε, 7840) corresponds to an endocyclic double bond conjugated with the lactone. Its N.M.R. spectrum did not indicate the presence of vinyl protons. The infrared spectrum had a broad band at 1745 cm^{-1} (γ-lactone and cyclopentanone) and a weak band at 1680 cm^{-1} (C—C double bond). Boron trifluoride isomerization of dihydroestafiatin (III) yielded the ketone (X) which formed an orange 2,4-dinitrophenylhydrazone. Catalytic hydrogenation of the ketone (X) afforded tetrahydroestafiatone (XI). This ketone yielded a crystalline cycloethylenemercaptol.

[11] E. A. Braude and E. R. H. Jones, *J. Chem. Soc.* 498 (1945); J. D. Roberts and C. Green, *J. Amer. Chem. Soc.*, **68**, 214 (1946); C. Djerassi and E. Ryan, *Ibid.* **71**, 1000 (1949).

There remains only to determine the orientation of the lactone. We could obtain evidence that the lactone is closed at C_6 on the following grounds. Alkaline treatment of the dihydronorketone (VI) did not produce an α,β-unsaturated ketone, which should be the case if the potential hydroxyl group of the lactone were at C_8. We obtained from this reaction an isomer which probably differs from VI in one of the asymmetrical centers at C_1 and C_{11} or in both. Lithium aluminum hydride reduction of the mixture of tetrahydroestafiatins followed by pyrolysis with selenium yielded artemazulene albeit in low yield.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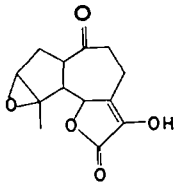
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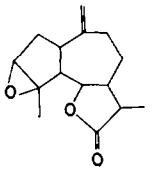
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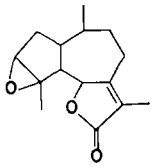
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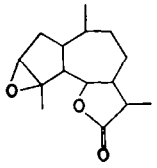
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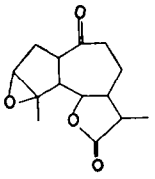
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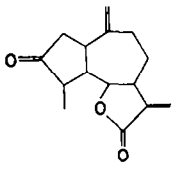
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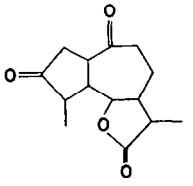
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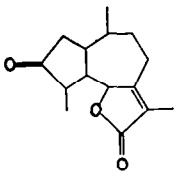
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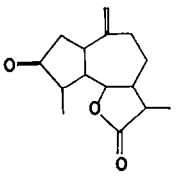
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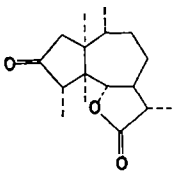
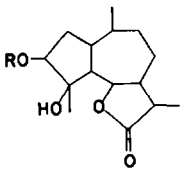

XI



XII
a R=H
b R=Ac

We could get further evidence concerning the structure of estafiatin and knowledge about its stereochemistry when it was correlated with isophoto-α-santonic lactone. Tetrahydroestafiatone XI, proved to be identical with a ketone derived from isophoto-α-santonic lactone obtained by Barton, Levisalles and Pinhey[12] in which the asymmetrical centers at C_4, C_5, C_6, C_7 and C_{11} are known. Furthermore the optical rotatory dispersion curve of tetrahydroestafiatone XI showed an intense, positive Cotton effect of the same type exhibited by 2 keto-A-norcholestane[13,14] which suggests a *trans* ring junction. The above correlation permits assignment of definite configuration to three of the six asymmetrical centers of estafiatin at C_5, C_6 and C_7.

[12] D. H. R. Barton, J. E. D. Levisalles and J. T. Pinhey, *J. Chem. Soc.* 3472 (1962). We are grateful to Dr. Barton for a sample of the ketolactone (numbered X in his paper).
[13] C. Djerassi, R. Riniker and B. Riniker, *J. Amer. Chem. Soc.* **78**, 6362 (1956).
[14] C. Djerassi, J. Osiecki and W. Herz, *J. Org. Chem.* **22**, 1361 (1957).

EXPERIMENTAL[15]

Isolation of estafiatin (I). *Artemisia mexicana* was collected in November, 1960, in the vicinity of Mexico City. The dried whole plant (10 K) was extracted with chloroform (40 l.) for 12 hr. The extract was filtered and evaporated to dryness. The residue was dissolved in methanol (2 l.) and a solution of lead acetate (60 g) in water (2 l.) was added, the mixture was allowed to stand overnight. The pale orange solution was then filtered and thoroughly extracted with chloroform. The extract was evaporated to dryness and submitted to steam distillation, to eliminate a volatile essential oil. The dried oily residue (35 g) was dissolved in 2 l. benzene, the same volume of hexane was added. The solution was filtered (to eliminate an amorphous material) and chromatographed on alumina (700 g). Several fractions eluted with benzene–hexane 1:1 crystallized; they were combined and by recrystallization from ether–hexane afforded 3·5 g estafiatin, m.p. 102°. Further crystallizations from hexane–pentane yielded needles m.p. 104–106°, $[\alpha]_D$ −9·9°; λ_{max} 214 mμ; ε, 9850); ν_{max} 1755, 1668, 1640 and 903 cm^{-1}. (Found: C, 72·96; H, 7·81; O, 19·33. Calc. for $C_{15}H_{18}O_3$: C, 73·14; H, 7·37; O, 19·49%). C-methyl 0·9 mol; mol. wt. (Rast) 265.

The fractions eluted from the chromatogram with benzene-hexane 2 : 1, 3 : 1, 4 : 1, and with benzene did not crystallize, several fractions were rechromatographed but crystalline material could not be isolated, they showed chemical and spectroscopic evidence of the lactone group.

Pyrazolines of estafiatin (I). A solution of estafiatin (100 mg) in ether (5 ml) was mixed with 30 ml of an ethereal solution of diazomethane (prepared with 1 g N-nitrosomethyl-urea) and allowed to stand overnight. Fractional crystallization from ether–hexane furnished plates (50 mg) m.p. 120° (dec); $[\alpha]_D$ +352°. Found: C, 66·52; H, 7·11; O, 16·45; N, 10·08. Calc. for $C_{16}H_{20}O_3N_2$: C, 66·64; H, 6·99; O, 16·65; N, 9·72%).

From the mother liquors, there was obtained other isomer m.p. 148–150° (dec) (needles from ether–hexane). (Found: C, 66·91; H, 7·09; O, 16·97; N, 10·09. Calc. for $C_{16}H_{20}O_3N_2$: C, 66·64; H, 6·99; O, 16·65; N, 9·72%).

Ozonolysis of estafiatin (I). A solution of the lactone (200 mg) in tetrahydrofuran (30 ml) was ozonized for 15 min at −70° (a large excess of ozone was passed through the solution), water (5 ml) was added and most of the solution was distilled into an aqueous solution of dimedone (200 mg) upon concentration of this solution to a small volume there crystallized 40 mg formaldehyde-dimedone, m.p. 189–190°, which showed no depression in mixed m.p. on admixture with an authentic specimen. In another experiment with the same amount of estafiatin, after ozonization, the solution was hydrogenated with 10% palladium on calcium carbonate (60 mg) until no more hydrogen was consumed. The catalyst was filtered and the solution evaporated to a small volume. Addition of ether crystallized the bisnorestafiatedione (II) (95 mg), m.p. 202–203°, further recrystallizations from acetone–ether gave the analytical sample, m.p. 208–210° (dec); $[\alpha]_D$ +53·6 (in dioxane), it showed a purple colour with ferric chloride, λ_{max} 236 mμ; ε, 10,700; ν_{max} 3500, 1750 and 1705 cm^{-1}. (Found; C, 62·18; H, 5·50; O, 32·18; Calc. for $C_{13}H_{14}O_5$: C, 62·39; H, 5·64; O, 31·97%).

Chlorohydrin of estafiatin (I). A solution of estafiatin (250 mg) in chloroform (5 ml) was mixed with 10 ml chloroform saturated with hydrogen chloride. The solution was left overnight at 4°, washed then with water and evaporated *in vacuo* to dryness. By crystallization of the oily residue from ether there was obtained the chlorohydrin, as needles (30 mg) m.p. 225° (dec); λ_{max} 214 mμ; ε, 8,500; ν_{max} (CCl_4), 3580, 1783, 1668. 1642 and 902 cm^{-1}. (Found: C, 63·79; H, 6·61; O, 17·04; Cl, 12·22. Calc. for $C_{15}H_{19}O_3Cl$: C, 63·71; H, 6·73; O, 16·99; Cl, 12·57%).

Dihydroestafiatin (III), *by chemical reduction.* To a solution of estafiatin (500 mg) in 60 ml ethanol, was added aluminium amalgam (freshly prepared; 1·5 g). The mixture was refluxed for 8 hr. The solution was filtered, concentrated to a small volume and extracted with ether, the ethereal solution was washed with water, dried (Na_2SO_4) and evaporated to dryness. Crystallization from pentane afforded 410 mg prismatic needles, m.p. 80–81°, $[\alpha]_D$ −4·7°, λ_{max} 205 mμ; ε, 3000; ν_{max} 1760, 1640 and 900 cm^{-1}. (Found: C, 72·33; H, 8·05; O, 20·00; Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12; O, 19·33%). C-methyl: 2 moles.

[15] M.p.s are uncorrected, rotations were determined at 20° in chloroform. The I.R. spectra were run in chloroform solution on a Perkin-Elmer double beam spectrophotometer. The U.V. absorption spectra were determined in 95% ethanol solution in a Beckman DK2 spectrophotometer unless noted otherwise. The microanalyses, C-methyl values and molecular weight determination were performed by Dr. Franz Pascher, Bonn, Germany.

By catalytic hydrogenation. A solution of estafiatin (750 mg) in ethyl acetate (20 ml) was hydrogenated with 10% palladium on calcium carbonate catalyst (150 mg) until one equivalent of hydrogen was absorbed. The catalyst was filtered and the solvent evaporated to dryness. The oily residue crystallized from pentane, yielding needles (450 mg) m.p. 74–76°, further crystallizations raised the m.p. to 78°. It showed no depression on mixed m.p. determination with the dihydroderivative obtained in the chemical reduction and the I.R. spectra were identical. Chromatography of the mother liquors on alumina (6 g) furnished in the fractions eluted with benzene hexane 1:1, dihydroestafiatin (III) m.p. 74–75° (40 mg) whereas from the fractions eluted with benzene–hexane 2:1 and 3:1 the new dihydro derivative (IV) was obtained. Crystallization from ether–hexane of the combined crystalline fractions yielded plates (30 mg) m.p. 152–154°, $[\alpha]_D$ +29°; λ_{max} 218 mμ; ε, 10,800; ν_{max} 1740 and 1668 cm^{-1}. (Found: C, 72·45; H, 8·08; O, 19·10; Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12; O, 19·33%).

Ozonolysis of dihydroestafiatin (III). The reaction was carried out as described in the previous experiments. There was obtained formaldehyde-dimedone (75 mg) m.p. 187–189°. In other reaction with dihydroestafiatin (230 mg), the solution of the ozonide was hydrogenated and worked up in a similar way. Crystallization from ether afforded nordihydroestafiatone (VI) as needles m.p. 142–145°, further crystallizations from acetone–ether raised the m.p. to 152–154°, $[\alpha]_D$ +2·3°; λ_{max} 280–282 mμ; ε, 80. ν_{max} 1770 and 1700 cm^{-1}. (Found: C, 66·87; H, 7·27; O, 25·95. Calc. for $C_{14}H_{18}O_4$: C, 67·18; H, 7·25; O, 25·57%).

Catalytic hydrogenation of estafiatin (I). A solution of estafiatin (1 g) in ethyl acetate (50 ml) was hydrogenated with Adams catalyst (100 mg) until no more hydrogen was absorbed. Two equivalents of hydrogen were consumed. The oily residue crystallized from hexane yielding tetrahydroestafiatin (a); (350 mg) m.p. 85–99°, the analytical sample was obtained by recrystallization from hexane as plates m.p. 104–106°, $[\alpha]_D$ +47·4°; ν_{max} 1765 cm^{-1}. (Found: C, 71·91; H, 8·96; O, 19·02. Calc. for $C_{15}H_{22}O_3$: C, 71·97; H, 8·86; O, 19·17%).

The mother liquors were chromatographed through 14 g alumina, several fractions eluted with hexane crystallized. They were combined recrystallized from hexane yielding tetrahydroestafiatin (25 mg) m.p. 100–101°. The analytical sample showed m.p. 103–105° (plates from hexane), $[\alpha]_D$ +27°, ν_{max} 1760 cm^{-1}. It gives depression in mixed m.p. with tetrahydroestafiatin (a) and the I.R. spectra were different. (Found: C, 72·11; H, 8·88; O, 19·26. Calc. for $C_{15}H_{22}O_3$: C, 71·97; H, 8·86; O, 19·17%).

The crystalline fractions eluted with hexane–benzene 4:1, 3:1 and 2:1 afforded tetrahydroestafiatin (c); (30 mg) m.p. 144–146° (needles from ether–hexane), $[\alpha]_D$ −38°; ν_{max} 1760 cm^{-1}. (Found C, 71·79; H, 8·91; O, 19·32, Calc. for $C_{15}H_{22}O_3$: C, 71·97; H, 8·86; O, 19·17%).

Catalytic hydrogenation of dihydroestafiatin (III). The dihydroderivative (300 mg) in 20 ml ethyl acetate with 40 mg Adams catalyst, after the uptake of one mole hydrogen, the adsorption ceased. The catalyst was filtered and the solvent evaporated to dryness. Crystallization from hexane yielded tetrahydroestafiatin (a); (240 mg) m.p. 96–97°. It proved to be identical with that obtained in the hydrogenation of estafiatin (I).

Estafiatone (VII). Estafiatin (300 mg) was dissolved in 15 ml anhydrous benzene and 6 drops of boron trifluoride etherate were added. The solution was left at room temp for 12 hr. It was washed with saturated sodium bicarbonate solution and water. Upon concentration of the solution there crystallized estafiatone (VII; 235 mg) m.p. 140–143°. The analytical sample showed m.p. 142–143° (needles from acetone–hexane), $[\alpha]_D$ −120°, estafiatone gave a positive Zimmermann test. λ_{max} 211 mμ; ε, 9460, ν_{max} 1760: 1740, 1668, 1640 and 900 cm^{-1}. (Found: C, 72·91; H, 7·37; O, 19·62. Calc. for $C_{15}H_{18}O_3$: C, 73·14; H, 7·37; O, 19·49%).

The 2,4-dinitrophenylhydrazone showed m.p. 225–226° (orange prisms from chloroform–methanol $[\alpha]_D$ +211·6°, λ_{max} ($CHCl_3$) 362–364 mμ; ε, 24,100). (Found: C, 59·16; H, 5·22; O, 22·64; N, 13·01; Calc. for $C_{21}H_{22}O_6N_4$: C, 59·15; H, 5·20; O, 22·51; N, 13·14%).

Hydrogenation of estafiatone (VII). A solution of estafiatone (235 mg) in acetic acid (10 ml) was hydrogenated with Adams catalyst (40 mg) until the absorption of hydrogen ceased. The catalyst was filtered and the solution evaporated *in vacuo* to dryness. The residue was crystallized from acetone–hexane yielding the ketone IX (180 mg) m.p. 203–206°, further recrystallizations from methanol raised the m.p. to 213–217°, $[\alpha]_D$ −11°, λ_{max} 220 mμ; ε, 7840; ν_{max} 1772, 1742 and 1680 cm^{-1}. (Found: C, 72·42; H, 8·36; O, 19·33; Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12; O, 19·33%).

Nordihydroestafiatedione (VIII). Nordihydroestafiatin (VI) (50 mg) was dissolved in benzene (4 ml) and 3 drops of boron trifluoride etherate were added. It was proceeded with as in previous case.

The diketone (VIII; 20 mg) showed m.p. 184–185° ν_{max} 1790, 1750 and 1718 cm^{-1}. (Found: C, 66·95; H, 7·40. Calc. for $C_{14}H_{16}O_4$: C, 67·18; H, 7·25%).

Dihydroestafiatone (X). Dihydroestafiatin (III); (100 mg) in benzene (5 ml) and 3 drops of boron trifluoride etherate was left 12 hr at room temp, the deep violet solution was worked up as in previous cases. Crystallizations from hexane afforded prisms (40 mg), m.p. 85–98°; $[\alpha]_D$ +139°, ν_{max} 284–294 mμ; ε, 68; λ_{max} 1780, 1750 and 1655 cm^{-1}. (Found: C, 72·60; H, 8·31; O, 19·45; Calc. for $C_{15}H_{20}O_3$: C, 72·55; H, 8·12; O, 19·33%).

The 2,4-dinitrophenylhydrazone showed m.p. 193–194° (orange needles from chloroform-methanol), $[\alpha]_D$ +229°; λ_{max} (in $CHCl_3$) 362–364 mμ; ε, 26,000. (Found: C, 58·57; H, 5·78; O, 22·62; N, 12·91. Calc. for $C_{21}H_{24}O_6N_4$: C, 58·87; H, 5·65; O, 22·41; N, 13·08%).

Tetrahydroestafiatone (XI). A solution of dihydroestafiatone (X); (250 mg) in ethanol (20 ml) was hydrogenated with Adams catalyst (40 mg), the absorption of hydrogen ceased after the uptake of one mole. The catalyst was filtered and the solution evaporated to dryness. The residue crystallized from acetone–hexane yielding prisms (180 mg), m.p. 177–188°. Further crystallizations from acetone–hexane raised the m.p. to 198°; $[\alpha]_D$ +82°. Tetrahydroestafiatone showed no depression in mixed m.p. determination with the derivative X of isophoto-α-santonic lactone and the I.R. spectra were identical. (Ref. 12).

Rotatory dispersion in dioxane $[\alpha]_{700}$ +40·6°, $[\alpha]_{589}$ +95·9°, $[\alpha]_{314}$ +2730°, $[\alpha]_{311}$ +2495°, $[\alpha]_{307\cdot5}$ +2545° (inflexion), $[\alpha]_{305}$ +2401°. (Found: C, 71·84; H, 9·08; O, 19·21. Calc. for $C_{15}H_{22}O_3$: C, 71·97; H, 8·86; O, 19·17%).

Cycloethylenemercaptol of tetrahydroestafiatin. To a solution of tetrahydroestafiatone (XI); (50 mg) in acetic acid (4 ml) were added 0·3 ml ethanedithiol and four drops of boron trifluoride etherate. The mixture was left at room temp overnight; diluted then with chloroform, washed with sodium bicarbonate solution and with water. The chloroformic solution was evaporated to dryness the oily residue crystallized from hexane yielding needles (45 mg) m.p. 153–154°. Further crystallizations from acetone–hexane raised the m.p. to 156°, $[\alpha]_D$ +18·7°. (Found: C, 62·52; H, 8·18; O, 10·08; S, 19·68; Calc. for $C_{17}H_{26}O_3S_2$: C, 62·56; H, 8·03; O, 9·80; S, 19·61%).

Alkaline treatment of tetrahydroestafiatin (a); (V). The tetrahydroderivative (a); (90 mg) was dissolved in methanol (5 ml) and mixed with a solution of potassium hydroxide (50 mg) in water (2 ml) and refluxed for 20 min. The solution was diluted with water (8 ml) acidified with dil. hydrochloric acid and extracted with chloroform. Crystallization from ether yielded the glycol (XIIa); (30 mg), m.p. 164–165°, $[\alpha]_D$ −15°; ν_{max} 3590 and 1762 cm^{-1}. (Found: C, 66·76; H, 8·95; O, 24·08; Calc. for $C_{15}H_{24}O_4$: C, 67·13; H, 9·01; O, 23·86%).

The monoacetate (XIIb); (acetic anhydride and pyridine, 1 hr on the steam bath) showed m.p. 137–139°; $[\alpha]_D$ −49°, ν_{max} 3570, 1780 and 1740 cm^{-1}. (Found: C, 65·79; H, 8·33; O, 25·65; Calc. for $C_{17}H_{26}O_5$: C, 65·78; H, 8·44; O, 25·78%).

Alkaline treatment of nordihydroestafiatone (XI). The ketone (VI) (25 mg) in methanol (5 ml) was treated with 3 ml of a 1% aqueous solution of potassium hydroxide. The solution was allowed to stand at room temp for 15 min, acidified then with acetic acid, diluted with water and extracted with chloroform. The chloroformic solution was washed with water and evaporated to dryness. By crystallization from ether there was obtained a ketone isomeric with VI (15 mg), m.p. 149–150°. The analytical sample showed m.p. 150–152° (needles from ether). It showed depression in mixed m.p. with nordihydroestafiatone (VI) and the I.R. spectra were different. ν_{max} 1770 and 1700 cm^{-1}. (Found: C, 66·95; H, 7·42; O, 25·79. Calc. for $C_{14}H_{18}O_4$: C, 67·18; H, 7·25; O, 25·57%).

Aromatization of tetrahydroestafiatin (X). The oily mixture of tetrahydroestafiatins (1·87 g) with 7 g selenium powder was heated at 280–290° for 20 min. The reaction mixture was extracted with hexane and chromatographed on alumina. The blue solution was evaporated to dryness and purified as the phosphoric acid complex. Diluted then with cold water and extracted with hexane, the hexanic extract was evaporated to dryness. The blue residue (40 mg) was dissolved in absolute ethanol and 40 mg trinitrobenzene was added. The reaction mixture was concentrated, there crystallized long black needles (25 mg) m.p. 123–126°. Further recrystallizations from ethanol raised the m.p. to 130–133°. It showed no depression in mixed m.p. with an authentic sample of chamazulene. T.N.B. adduct. The I.R. spectra were identical.

When the mixture of tetrahydroestafiatins (0·5 g) was reduced with lithium aluminium hydride (0·5 g) in ether (30 ml) for 6 hr and then pyrolyzed with selenium (1·5 g), there was obtained artemazulene, characterized as its T.N.B. adduct, m.p. 190° (black needles, 4 mg, crystallized from ethanol). It was identified with an authentic sample by the standard methods.