methanol in ether (2 l.) and 5% methanol in ether (0.5 l.) provided, after evaporation to dryness, a grayish gum which solidified upon trituration with ether (XIX, 387 mg). The solid product showed λ_{max} 2.80, 2.90 (broad and intense), 5.78, 6.00, 6.20 μ; λ_{max} 236 mμ.

The mixture of diols was then utilized as such.

Δ^4-Etiojervene-3,11,17-trione (XXI).—A mixture of the epimeric diols (XIX, 25 mg) was oxidized with chromic acid (20 mg) in glacial acetic acid (10 ml) and water (1 ml) at room temperature for a period of 4 hr. The reaction mixture was poured over cracked ice and was extracted with chloroform (30 ml). The chloroform layer was washed with a saturated sodium bicarbonate solution, water, and was then dried over anhydrous sodium sulfate. Evaporation of the chloroform gave a brownish solid which was crystallized from acetone–petroleum ether to provide needles of XXI: mp 214–216°; λ_{max} 5.78, 5.85, 6.00, 6.20 μ (superimposable upon that of the authentic sample); λ_{max} 236 mμ (ϵ 15,500); mixture melting point with the authentic sample was undepressed.

Δ^4-17-Ethyletiojerven-17α-ol-3,11,20-trione (C-Nor-D-homo-11-keto-17α-hydroxyprogesterone) (XX) and Δ^4-17-Ethyletiojerven-17β-ol-3,11,20-trione (C-Nor-D-homo-11-keto-17β-hydroxyprogesterone) (XXII). Method A.—To a stirred solution of XI (700 mg, mp 164–165°) in *t*-butyl alcohol under a nitrogen atmosphere was added a solution of 1.5 *M* H_2O_2 (4 ml) in *t*-butyl alcohol (5 ml), followed by a solution of osmium tetroxide (25 mg) in *t*-butyl alcohol (10 ml) which was added slowly over a period of 1 hr. Stirring was continued for 48 hr, after which was added a solution of sodium sulfite (0.7 g) in water (5 ml) and the stirring was continued for an additional 15 min. Water was added and the reaction mixture was extracted with ether (0.5 l.). The ethereal extract was washed with water and dried over anhydrous sodium sulfate. Evaporation of the ether to dryness gave a solid (0.95 g).

Column chromatography of the residue on silica gel (30 g) packed in benzene and elution with chloroform–benzene mixtures (up to 50% $CHCl_3$, 2 l.) gave starting material (300 mg). Further elution with chloroform–benzene (1:1, 1 l.) provided, after evaporation to dryness, a white solid (280 mg, mp 208–212°) composed mainly of XX. Crystallization from chloroform–petroleum ether gave white needles: mp 215–216°; $[\alpha]^{26}D$ +54° (*c* 0.52); λ_{max} 2.85 (weak), 5.78, 5.84, 6.00, 6.20 μ; λ_{max} 236 mμ (ϵ 17,100); ORD $[\phi]_{270}^{peak}$ +17411°; $[\phi]_{332}^{trough}$ −17360°; a = −347°.

Anal. Calcd for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 73.28; H, 8.45.

Continued elution with chloroform (1 l.) yielded a white solid (250 mg, mp 225–232°) composed mainly of XXII. Crystallization from chloroform–petroleum ether afforded colorless plates: mp 230–235° dec; $[\alpha]^{27}D$ +56° (*c* 0.43); λ_{max} 2.80, 2.90 (broad and intense), 5.78, 5.84 (moderate), 6.00, 6.20 μ; λ_{max} 236 mμ (ϵ 18,000); ORD $[\phi]_{280}^{peak}$ +7021°; $[\phi]_{328}^{trough}$ −12287°; a = −193°.

Anal. Calcd for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 73.48; H, 8.39.

Oxidation of XII (60 mg) according to the procedure above yielded three major bands when chromatographed on preparative tlc on silica gel F. The bands were extracted with 1% methanol in chloroform. Band I yielded starting material (20 mg). Band II yielded pure XX (18 mg, mp 215–216°) after crystallization from chloroform–petroleum ether. Band III yielded pure XXII (25 mg) after crystallization from chloroform–petroleum ether.

Method B.—To a solution of potassium chlorate (100 mg) and a catalytic amount of osmium tetroxide (5–10 mg) in tetrahydrofuran (10 ml) and water (5 ml) was added a solution of XI (150 mg, mp 164–165°) in tetrahydrofuran (5 ml). The solution was maintained at 48–50° for a period of 24 hr (or until the disappearance of XI, followed by tlc). Water was added and the resulting precipitate was extracted with ether (200 ml) and the ethereal extract was washed with water and dried over anhydrous sodium sulfate. Evaporation of the ether gave a grayish gum composed mainly of XIX (155 mg), which was dissolved in pyridine (10 ml) and treated with a solution of chromic acid (80 mg) in pyridine (10 ml). The reaction mixture was allowed to stand at room temperature for 30 min and refrigerated for an additional 18 hr. This was poured over cracked ice and water was added before extraction with chloroform (100 ml). The chloroform layer was washed successively with 5% hydrochloric acid solution, 5% sodium bicarbonate solution, and water, and was then dried over anhydrous sodium sulfate. The chloroform was evaporated to dryness to leave a brown residue (120 mg), which was chromatographed on acid-washed alumina (4 g) packed in benzene. Elution with 30% ether in benzene (200 ml) gave an oil (40 mg, λ_{max} 5.78, 6.00, 6.20 μ; λ_{max} 249 mμ) which failed to crystallize. This was believed to be the dehydrated product, $\Delta^{4,16}$-17-ethyletiojervadiene-3,11,20-trione (XVIII). Further elution with 40% ether in benzene gave a solid (25 mg) which crystallized from chloroform–petroleum ether as white needles (XX, mp 215–216°). Continued elution with ether (400 ml) gave a solid (27 mg) which also crystallized from chloroform–petroleum ether to provide colorless plates of XXII, mp 230–234° dec.

Registry No.—IV, 15285-77-5; V, 15285-78-6; VI, 15285-79-7; VII, 15285-80-0; VIII, 15285-81-1; XI, 15285-66-2; XII, 15285-67-3; XIII, 15285-68-4; XIV, 15285-69-5; XV, 15285-70-8; XVI, 15285-71-9; XVII, 15285-72-0; XVIII, 15285-73-1; XIX, 15285-74-2; XX, 15314-05-3; XXI, 15285-75-3; XXII, 15285-76-4.

Sesquiterpene Lactones of *Encelia farinosa* Gray[1]

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Two sesquiterpene lactones isolated from *Encelia farinosa* Gray (Compositae, tribe Heliantheae), farinosin and encelin, have been found to have the structures **2** and **5**, respectively. Encelin, dehydrofarinosin, is also obtained by dehydration of farinosin. Farinosin is the principal lactonic constituent of the leaves while encelin is found in the green stems. As the plant becomes senescent and scarious the compounds disappear.

Encelia farinosa Gray (tribe Heliantheae) is a composite abundant on the southwestern deserts. The plant is a perennial, flowering in March and April. The leaves and stems of early spring growth contain components that are characterizable as sesquiterpene lactones and which disappear as the temperatures rise in early summer and the plant becomes scarious. An examination of *E. farinosa* was undertaken because relatively little study has been devoted to members of the Heliantheae, of which members of the widely studied genus *Ambrosia* have been designated as a subtribe.[2]

Two sesquiterpene lactones, farinosin and encelin, have been isolated from *E. farinosa*. The former is the principal component of the leaves, which contain but traces of encelin; the latter is present in the green stems. Farinosin, mp 200–201°, has the composition

(1) Contribution No. 2151 from the Department of Chemistry, U.C.L.A.

(2) P. A. Munz and D. D. Keck, "A California Floria," University of California Press, Berkeley, 1959, p 1100. However, W. L. Jepson ("A Manual of the Flowering Plants of California," Associated Students Store, University of California, Berkeley, 1923) confers tribal status (Ambrosieae) upon the ragweeds.

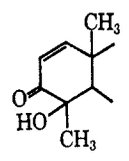
TABLE I

NMR CHEMICAL SHIFTS OF PROTONS OF FARINOSIN AND ITS DERIVATIVES[a]

	Proton positions										
	CH at		CH₃ at			=CH₂ at			OCOCH₃	OH	CH
Compd	1	2	4	10	11	4		11	at 4	at 4	at 8
2	6.85 (d)	6.02 (d)	1.52 (s)	1.00 (s)	...	...	...	6.15 (d) 5.25 (d)	...	2.87 (s)	5.07 (m)
	J = 10[b]							J = 3			
3	7.14 (d)	5.92 (d)	1.32 (s)	1.18 (s)	...	...	...	... ...	...	2.83 (s)	5.02 (m)
	J = 10										
4	6.82 (d)	6.00 (d)	1.70 (s)	0.99 (s)	...	...	...	6.15 (d) 5.25 (d)	2.08 (s)	...	4.85 (m)
	J = 10							J = 3			
5[c]	6.85 (d)	6.05 (d)	...	1.04 (s)	...	6.21 (d) 5.70 (d)		6.17 (d) 5.25 (d)	...	...	4.62 (m)
	J = 10					J = 3		J = 3			
6[c]	7.20 (d)	5.97 (d)	...	1.26 (s)	...	...	...	... ...	...	...	5.57 (m)
	J = 10										
7[c]	...	...	1.38 (s)	1.16 (s)	0.99 (d)	...	...	... ...	...	2.85 (s)	4.94 (m)
					J = 7						
8	...	...	...	1.20 (s)	1.02 (d)	6.16 (d) 5.64 (d)		... ...	...	...	4.54 (m)
					J = 7	J = 3					
9	...	...	1.22 (d)	1.18 (s)	1.04 (d)	...	...	... ...	...	...	4.50 (m)
			J = 7		J = 7						

[a] Shifts (parts per million) measured in $CDCl_3$ (except where otherwise specified) on a Varian A-60 spectrometer with tetramethylsilane as the internal reference: s, singlet; d, doublet; and m, multiplet. [b] Coupling constants in cycles per second. [c] Measured in deuterioacetone.

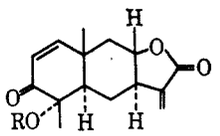
$C_{15}H_{18}O_4$ and its mass spectrum shows the expected molecular ion peak at m/e 262. The infrared spectrum contains strong peaks at 3280 (OH), 1770 (γ-lactone), 1658 (cyclohexenone), 1610, and 850 cm^{-1}. It shows ultraviolet absorption at 239 mμ (ϵ 10,090), indicating the presence of an α,β-unsaturated ketone grouping. The nmr spectrum (Table I) reveals the characteristic pair of doublets (δ 5.25 and 6.15, 1 H each, J = 3 cps) of a methylene group conjugated with the lactonic carbonyl group. A pair of doublets having the characteristic AB pattern of a cyclohexenone are found at δ 6.02 and 7.85 (1 H each, J = 10 cps). That these protons show no additional splitting and the appearance of a three-proton singlet at δ 1.00 suggested that the double bond is at C-2–C-3 of a eudesmanolide, which bears a methyl group at C-10. These observations, coupled with the presence of a sharp three-proton singlet at δ 1.52 (tertiary methyl) and a one-proton singlet at δ 2.87, characteristic of a tertiary hydroxyl group, lead to the conclusion that farinosin contains the partial structure **1**.



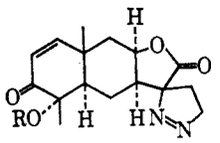
1

The position of closure of the lactone ring can be inferred from the nmr signal for the proton on the carbon atom holding the lactonic oxygen atom. That this carbon atom is at C-8 (rather than the alternative C-6) is shown by the multiplicity of the nmr signal at δ 5.07, the appearance of which is consonant only with spin coupling of the proton with three adjacent hydrogen atoms. Indeed, the signal strongly resembles that for the corresponding proton in yomogin,[3] which possesses a *cis*-C_7–C_8 lactone. Estimated coupling constants for this proton in farinosin are nearly the same as those found for yomogin.

The presence of the structural features revealed by the spectral data was confirmed by chemical evidence. Farinosin readily formed a red 2,4-dinitrophenylhydrazone whose ultraviolet absorption spectrum (λ_{max} 257, 387 mμ; ϵ 14,200 and 29,000) is characteristic of the derivative of a cyclohex-1-en-3-one. Treatment of farinosin **2** with diazomethane yielded the monopyrazoline **3**, in the nmr spectrum of which there no longer appeared the signals for the protons of the exocyclic methylene group.[4] The signal for the C-10



2, R = H
4, R = $COCH_3$



3

methyl group now appears at δ 1.18, while that for the methyl group at C-4 is seen at δ 1.32. These shifts are consistent with the indicated (relative) stereochemistry at positions 4, 7, and 10. The mass spectrum of the pyrazoline shows a weak molecular ion peak (m/e 304), the most intense (base) peak appearing at m/e 276 (M − 28). Important peaks at m/e 261, 248, and 217 correspond to the loss of nitrogen plus CH_3, CO, and CO_2, respectively.

Farinosin, although a tertiary alcohol, readily formed a crystalline acetate, **4**. The ease of acetylation is entirely in accord with the structure shown and can be attributed to the location of the hydroxyl group adjacent to the ketonic carbonyl group. For example, Nakanishi, *et al.*,[5a] found that isoilludin S, an α-ketol with a tertiary hydroxyl group, readily forms the corresponding acetate. Farinosin acetate shows the three-proton singlet for the acetoxy methyl group (δ 2.08), while the signal for the hydroxyl proton, seen in the spectrum of farinosin, has disappeared. The placing of the hydroxyl group at C-4 is further con-

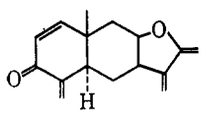
(3) T. A. Geissman, *J. Org. Chem.*, **31**, 2523 (1966).

(4) The indicated stereochemistry of ring A is supported by evidence to be described in discussion to follow.

(5) (a) K. Nakanishi, M. Ohashi, M. Tada, and Y. Yamada, *Tetrahedron*, **21**, 1231 (1956); (b) T. A. Geissman and G. A. Ellestad, *J. Org. Chem.*, **27**, 1855 (1962).

firmed by the shift in the signal for the methyl group at C-4 from δ 1.52 (in farinosin) to δ 1.70 in the acetate. The mass spectrum of **4** shows the molecular ion at *m/e* 304 and includes peaks at *m/e* 289 (M − 15), 244 (M − 60), 229 (M − 60 − 15).

Dehydration of farinosin yielded a single compound, dehydrofarinosin **5**, in excellent yield. Dehydrofarinosin proved to be identical with encelin, isolated from *E. farinosa*, in which it is the principal component of the stems. The nmr spectrum of encelin is identical with that of the product of dehydration of farinosin and is in accord with the structure shown. The signals for the C-4 methyl group and the hydroxyl group are absent and in their place appears a new pair of one-proton doublets at δ 5.70 and 6.10 (J = 3 cps), characteristic of the new exocyclic methylene group. The formation of the exocyclic methylene group is compelling evidence for the *cis* relationship between the hydroxyl group at C-4 and the C-5 hydrogen atom. Vulgarin,[5b] in which the C-4 hydroxyl group is disposed in the same way, undergoes dehydration to give mainly the exocyclic methylene group at C-4. If the stereochemistry of farinosin is as shown in **2**, the isomer formed by dehydration between C-4 and C-5 would be yomogin, which was sought among the products of the dehydration reaction but was not found. It was observed in control experiments that encelin and yomogin have identical R_f values on thin layer chromatograms; consequently, had traces of the latter been formed in the dehydration of farinosin, it would have been difficult to recognize its presence.

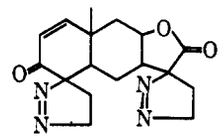


5

The mass spectrum of encelin[6] shows the molecular ion at *m/e* 244, along with peaks at *m/e* 229 (M − 15), 216 (M − 28), 200 (M − 44), 185 (M − 44 − 15). A peak at *m/e* 91[7] and one at *m/e* 105, of nearly equal intensities, represent base peaks and others peaks mentioned are of intensities of about 25–40% of these.

The most persuasive evidence for the presence of two carbonyl-conjugated exocyclic methylene groups in encelin is the ready formation of a bispyrazoline derivative, **6**, when encelin is treated with diazomethane. The nmr spectrum of the bispyrazoline derivative shows the loss of the four protons of the methylene groups of encelin; the only proton signals in the vinyl region are the well-defined doublets at δ 5.97 and 7.20 (J = 10 cps) for protons of the AB system at C-1 and C-2 of the cyclohexenone ring. The four protons of the CH_2 groups adjacent to N=N are seen in a multiplet centering at δ 4.58 and the proton at C-8 gives a multiplet at δ 5.57. A single three-hydrogen singlet for the C-10 methyl group appears at δ 1.24. The spectrum is in complete accord with structure **6**.

Reduction of farinosin with zinc and acetic acid yielded the tetrahydro compound **7**. The infrared spectrum (in chloroform) of **7** showed the lactone and cyclohexanone carbonyl groups at 1775 and 1715 cm^{-1}, respectively. The nmr spectrum showed but two protons at fields lower than δ 2.5, namely, a one-proton singlet (OH) at δ 2.80 and a one-proton multiplet (proton at C-8) at δ 4.90. The methyl groups at C-4 and C-10 appeared as sharp three-proton singlets at δ 1.38 and 1.16, respectively, and the methyl group of the lactone ring gave a doublet at δ 0.99 (J = 6.5 cps). The signal for the hydroxyl group at δ 2.80 (and for that of the hydroxyl group in farinosin) disappeared upon the addition of deuterium oxide. Although but one tetrahydrofarinosin was isolated as a pure, crystalline compound, the thin layer chromatogram of the crude reduction product showed spots at R_f 0.38 and 0.40, suggesting the presence of the two epimers (at C-11). The mass spectrum of tetrahydrofarinosin showed no molecular ion; the peak of highest *m/e* value was at 248 (M − 18). Other significant peaks were observed at *m/e* 222 (M − 44), 178, 163, 136, 121, along with the ubiquitous (in this series) peaks at 91 and 105.



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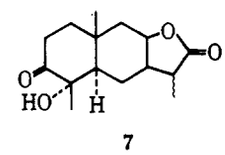

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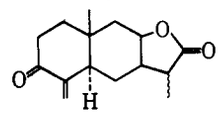

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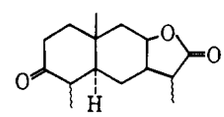

9

Dehydration of tetrahydrofarinosin yielded dehydrotetrahydrofarinosin, **8**; the ultraviolet spectrum of this showed a maximum at 210 mμ (ϵ 11,640) and infrared peaks were present at 1750 (lactone), 1690 (cyclohexanone), 1660 (C=C), and 825 cm^{-1}. The latter band has been ascribed to a carbonyl-conjugated exocyclic methylene group.[8] The nmr spectrum of **8** showed the C-8 proton at δ 4.59 (multiplet, 1 H) and two protons at δ 5.70 and 6.21 (1 H each, J = 2–3 cps), characteristic of the exocyclic methylene group of **8** and in the same position as those of the corresponding protons of encelin. The methyl group at C-4, present in tetrahydrofarinosin, is absent in the spectrum of **8**; only two methyl group signals are present, that for the methyl group at C-10 (δ 1.26, 3 H, singlet) and for the methyl group at C-11 (δ 1.02, 3 H, doublet, J = 7 cps). The mass spectrum of **8** showed the molecular ion at *m/e* 248 and other peaks at *m/e* values of 233 (M − 15), 220 (M − 28), 204 (M − 44), and 175 (M − 73).

Catalytic hydrogenation of **8** yielded the expected saturated ketone, **9**, which is one of the possible isomers of which 3-ketotetrahydroalantolactone is one. Two stereomers of this structure have been prepared from isotelekin,[9] one of which possesses a melting point in agreement with that of **9**. A specimen of this compound (XIb in ref 9) did not depress the melting point of **9**; the two compounds showed identical behavior on tlc.

The stereochemical features expressed in structure **2** include two assumptions, namely, that the A/B

(6) Because encelin was much less abundant in the plant than farinosin, most of the chemical manipulations were performed with material prepared by the dehydration of farinosin.

(7) The peak at *m/e* 91 is present in the mass spectra of all of the sesquiterpenoid lactones that have been studied in this laboratory. It probably represents the tropylium ion, $C_7H_7^+$. The *m/e* 105 peak, present in most of the mass spectra reported here, may be the methyltropylium ion, $C_7H_6CH_3^+$.

(8) M. M. Mehra, K. G. Deshpande, B. B. Ghatge, and S. C. Battacharya, *Tetrahedron*, **23**, 2469 (1967).

(9) V. Benesova, V. Herout, and F. Sorm, *Collection Czech. Chem. Commun.*, **26**, 1350 (1961).

ring junction is *trans* and the C-10 methyl group is β, as in such other members of this class of eudesmanolides such as vulgarin,[5] isotelekin,[9] arglanine,[10] douglanine,[11] santamarine,[12] pinnatifidin,[13] and tuberiferine,[14] and that the C-7–C-11 bond is β oriented.

Experimental Section

Melting points were taken in capillary tubes in a Swissco melting point bath and are corrected. Thin layer chromatograms were prepared with the use of Merck silica gel G, developed with chloroform–acetone (4:1) and visualized by spraying with concentrated sulfuric acid and heating. The nmr spectra were measured with a Varian A-60 instrument in deuteriochloroform with tetramethylsilane as an internal standard. Ultraviolet (in ethanol) and infrared (as noted) spectra were measured in the usual way. Mass spectra were recorded with an AEI MS-9 spectrometer operating at 70 ev by the direct insertion technique. The nmr data are collected in Table I and discussed in the earlier part of the paper.

Farinosin (2). Isolation from *Encelia farinosa*.—*Encelia farinosa* Gray was collected in March 1967, in the area of Palm Springs, Calif. Leaves were stripped from the plant, dried at 50°, ground in a Wiley mill, and extracted with chloroform at room temperature. The extract was processed in the manner previously described.[3] The final crude chloroform extract yielded a brown-yellow oil (6.5 g from 1 kg of dry plant material), which was chromatographed over 200 g of silica gel with chloroform as the eluent. Fractions containing farinosin (by tlc) were combined and upon evaporation yielded the crystalline lactone (yield 0.13% of the dry leaves). Recrystallized from chloroform–hexane, the compound formed colorless needles, mp 200–201°. It had R_f 0.32 in the tlc system described and $[\alpha]^{24}D$ −111° (*c* 2.25, $CHCl_3$).

Anal. Calcd for $C_{15}H_{18}O_4$: C, 68.70; H, 6.87. Found: C, 68.65; H, 6.92.

The relevant spectral (uv, ir, nmr, mass) characteristics have been described in the earlier discussion.

In a separate experiment, a sample of *E. farinosa* (whole plant) collected near Tuscon, Ariz., in June yielded only 0.005% of farinosin. This low yield is probably attributable in part to the low lactone content of the woody stems.

Farinosin 2,4-Dinitrophenylhydrazone.—The 2 2,4-dinitrophenylhydrazone was prepared in the usual way and was recrystallized from methanol as red leaflets, mp 266° dec. It had λ_{max} 257 mμ (ε 14,200) and 387 (29,000) and infrared ($CHCl_3$) maxima at 3310, 1760, 1613, 1584, and 840 cm^{-1}. It showed a molecular ion at *m/e* 442 in the mass spectrometer.

Anal. Calcd for $C_{21}H_{22}N_4O_7$ (442): C, 57.01; H, 5.01. Found: C, 56.96; H, 5.14.

Farinosinmonopyrazoline (3).—To a solution of 140 mg of farinosin in 25 ml of tetrahydrofuran was added a solution of diazomethane (from 400 mg of nitrosomethylurea) in ether, over a period of 1 hr. Another like portion of diazomethane was added the next day; after allowing the solution was allowed to remain overnight at 5° the excess diazomethane was destroyed and the solvent evaporated. The product was recrystallized from acetone and formed buff needles: mp 195–196° dec; $[\alpha]^{26}D$ −217° (*c* 0.71, methanol).

Anal. Calcd for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.21. Found: C, 63.35; H, 6.51; N, 9.16.

Infrared peaks (KBr) were observed at 3300, 1765, 1662, 1654, and 1550 cm^{-1} and the ultraviolet spectrum showed λ_{max} 228 mμ (ε 12,000) and 320 (257). The mass spectrum has been discussed above.

Farinosin Acetate (4).—A solution of 262 mg of farinosin in a mixture of 5 ml of acetic anhydride and 3 ml of pyridine was kept at 5° for 24 hr. The solvents were removed *in vacuo* and the crude product in chloroform was passed through a column of silica gel. The purified acetate (258 mg) was recrystallized from chloroform–hexane and formed colorless needles: mp 188–189°; R_f 0.60 in the tlc system described; $[\alpha]^{26}D$ −94° (*c* 2.8, $CHCl_3$). It showed λ_{max} 239 mμ (ε 10,160) and infrared bands ($CHCl_3$) at 1768, 1728, 1660, 1612, 1220, and 850 cm^{-1}. The nmr data are found in Table I.

Anal. Calcd for $C_{17}H_{20}O_5$: C, 67.09; H, 6.62. Found: C, 67.21; H, 6.43.

Dehydrofarinosin (5).—To a cold solution of 330 mg of farinosin in 10 ml of pyridine was added 1.5 ml of phosphorus oxychloride and the mixture was allowed to stand for 18 hr. The solvents were removed *in vacuo*, the solid residue was dissolved in chloroform, and the dried (Na_2SO_4) solution was passed through a column of silica gel. The product isolated from the eluate (242 mg) was recrystallized from ethyl acetate–hexane to give pure dehydrofarinosin as colorless needles: mp 195–196°; R_f 0.61; $[\alpha]^{26}D$ −16.5 (*c* 3.67, $CHCl_3$). Ultraviolet maxima were observed at 239 mμ (ε 10,570) and 210 (15,140). The infrared spectrum showed peaks at 1760, 1660, 1613, and 850 cm^{-1}.

Anal. Calcd for $C_{15}H_{16}O_3$: C, 73.75; H, 6.60. Found: C, 73.56; H, 6.83.

The compound was identical with encelin (see below).

Dehydrofarinosinbispyrazoline (6) was prepared as described for farinosinmonopyrazoline, except that twice the quantity of diazomethane was used. The compound was recrystallized from acetone–methanol as colorless flakes, mp 180–181° dec. It had R_f 0.37; $[\alpha]^{28}D$ 6.5° (*c* 0.69, methanol); ultraviolet maxima, 227 mμ (ε 14,150) and 322 (545). The infrared spectrum showed bands at 1760, 1660, 1605, and 1545 cm^{-1}. The mass spectrum showed no molecular ion, but prominent peaks at *m/e* 272 (M − 56) and 257 (M − 56 − 15), corresponding to the loss of N_2 and $N_2 + CH_3$.

Anal. Calcd for $C_{17}H_{20}N_4O_3$: C, 62.19; H, 6.10; N, 17.09. Found: C, 62.25; H, 6.21; H, 17.39.

Tetrahydrofarinosin (7).—A solution of 2 g of farinosin in 200 ml of acetic acid was heated under reflux while 10 g of zinc dust was added. After heating for 24 hr, the solution was filtered and the solvent evaporated. The crude product showed two spots on tlc at R_f 0.38 and 0.40. Upon repeated recrystallization from aqueous methanol the R_f 0.40 spot at length disappeared and pure tetrahydrofarinosin (244 mg) was obtained. It had mp 255–256°; R_f 0.38; $[\alpha]^{28}D$ −16.2° (*c* 0.74, methanol). It showed no high-intensity absorption in the ultraviolet region (a peak at 282 mμ (ε 30) corresponds to the saturated cyclohexanone moiety) and had the infrared maxima ($CHCl_3$) described earlier.

Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.65; H, 8.33. Found: C, 67.58; H, 8.36.

Dehydrotetrahydrofarinosin (8).—A solution of 150 mg of tetrahydrofarinosin in a mixture of 5 ml of pyridine and 0.75 ml of phosphorus oxychloride was kept for 18 hr and the product isolated by removal of the solvents, extraction of the residue with chloroform, and passage of the chloroform solution through a column of silica gel. The dehydro compound (115 mg) was recrystallized from chloroform–ether to give colorless needles: mp 218–219°; R_f 0.61; $[\alpha]^{28}D$ 52.2° (*c* 1.14, $CHCl_3$). The compound showed λ_{max} 210 mμ (ε 11,640) and infrared maxima at 1750, 1690, and 825 cm^{-1}.

Anal. Calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.42; H, 8.38.

4-Deoxytetrahydrofarinosin (9).—Hydrogenation of dehydroanhydrofarinosin (8) in ethyl acetate with a platinum catalyst resulted in the ready uptake of 1 mole of hydrogen. The crude product showed two spots on tlc (R_f 0.45 and 0.57), the higher R_f spot predominating. The crude reduction product, in chloroform, was passed through a column of silica gel and yielded the pure dihydro compound 9 (35 mg from 50 mg of 8): R_f 0.57; mp 173–174°; $[\alpha]^{28}D$ −31.8° (*c* 0.77, $CHCl_3$). A compound of the same gross structure, prepared from isotelekin, had a reported mp 171–172° and a stereoisomer had mp 182–183°.[9,15] A specimen of the lower melting isomer did not depress the melting point of 9 and the two samples showed identical behavior on tlc.[14] Compound 9 showed only the low-intensity band, λ_{max} 280 (ε 53), in the ultraviolet characteristic of the cyclohexanone unit and infrared peaks at 1760 and 1700 cm^{-1}. When a sample of 9 was warmed in solution in hydrochloric acid, the R_f remained unchanged, an indication that the 4-methyl group is indeed equatorially disposed. The compound, mp 171–172°, from isotelekin[9] is assigned the 4-CH_3 (equatorial) configuration.

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Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.88; H, 8.79.

Isolation of Encelin (Anhydrofarinosin from *E. farinosa*).—Thin layer chromatograms of the leaves, stems and whole plant of *E. farinosa* showed the presence of, respectively, a single spot of farinosin, a single spot of encelin, and the two spots. The yellow-green stems (including the naked peduncles) were extracted separately and the extract was processed in the usual way.[3] Chromatography of the final crude syrup, isolated by chloroform extraction (5 g from 1 kg of dry plant material), over silica gel (eluent, chloroform–methylene chloride, 1:1) and concentration of the fractions containing encelin (by tlc) yielded 350 mg (0.035%) of the compound. It had mp 195–196°, undepressed on admixture with anhydrofarinosin, and its spectral (uv, ir, nmr) characteristics were identical with those oft he latter.

Anal. Calcd for $C_{15}H_{16}O_3$: C, 73.75; H, 6.60. Found (from plant): C, 73.81; H, 6.84.

Senescent *E. farinosa*.—A collection of *E. farinosa* leaves was made in June, at which time the desert temperatures were in the range of 100°F and the plant had become gray and scarious. Thin layer chromatograms of extracts of the leaves showed the presence of much low-R_f material, but little or no farinosin.

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Microbiological Transformation of Steroids. I. The Synthesis of 19-Nortestololactone[1]

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The major pathway for the oxidative degradation of 19-nortestosterone by the fungus *Aspergillus tamarii* to produce 19-nortestololactone has been found to be analogous to that for testosterone. The consecutive steps to the D-ring δ-lactone involve the initial interaction of the steroid substrates with 17-ketodehydrogenase followed by the action of a lactonase enzyme system. An 11β-hydroxylase enzyme system was also found to be operative on the 19-nortestosterone substrate since 11β-hydroxy-19-nortestosterone, 19-norandrost-4-en-11β-ol-3,17-dione, and 11β-hydroxy-19-nortestololactone were obtained. The latter compounds were also isolated from fermentation of 11β-hydroxy-19-nortestosterone with *A. tamarii*, which apparently represents a contradiction to the published generality that *A. tamarii* lacks the ability to degrade oxidatively the D ring of 11-hydroxylated steroids. This is the first report of successful microbiological synthesis and characterization of a 19-nortestololactone.

Several paper[3-9] and review articles[10-14] have been published concerning the ability of fungi to degrade stereospecifically androstane(ene)- and pregnane(ene)-type steroids to their respective D-ring δ-lactone derivatives. Recent investigations[8,9,15-17] have been oriented toward elucidation of the oxidative mechanism by which δ-lactone formation occurs.

Capek and coworkers[5] have demonstrated the primary pathway in the production of testololactone from progesterone fermentation with *Aspergillus oryzae* to involve the production of testosterone and androst-4-ene-3,17-dione as sequential intermediates. The isolation of testosterone acetate as an intermediate product in the fermentation of progesterone with *Cladosporium resinae*[8] and the isolation of 20β-hydroxy-4-pregnen-3-one in the early hours of incubation of progesterone with *Penicillium lilacinum*[9] indicate still other potential intermediates in the biooxidation of progesterone. It was also reported that 11α-hydroxyprogesterone could be converted into 11α-hydroxytestololactone by *P. lilacinum via* 11α,20β-dihydroxy-4-pregnen-3-one, 11α-hydroxytestosterone, and 11α-hydroxyandrost-4-ene-3,17-dione.[9,19]

More recently, Brannon, *et al.*,[18] found that incubation of progesterone with *Aspergillus tamarii* gave rise not only to the expected testosterone, androst-4-ene-3,17-dione, and testololactone, but also produced 11β-hydroxytestosterone as a terminal by-product; however, formation of 11β-hydroxytestololactone or 11α-hydroxytestololactone by fermentation of 11-hydroxylated pregnenes and androstenes with *A. tamarii* did not occur. Consequently, Brannon, *et al.*,[18] concluded that the fungus *A. tamarii* is unusual in its inability to degrade oxidatively the D ring of 11-hydroxylated steroids.

Our work has been concerned with the interaction of 19-nortestosterone with *A. tamarii* to determine the effect of the absence of the 10β-methyl substituent. Incubation of 19-nortestosterone with *A. tamarii* for 72 hr gave five transformation products. Two of these products, 19-norandrost-4-ene-3,17-dione and 19-nortestololactone, apparently were derived from an oxidative pathway similar to that which has been proved for testosterone.

The D-ring δ-lactone structural assignment for previously unreported 19-nortestotolactone was chiefly derived from its nmr spectrum. Examination of the latter shows the C_{18} methyl signal to be at 82.5 cps. The corresponding signal for the starting material, 19-nortestosterone, has a value of 49.0 cps. These values represent a downfield chemical shift of 33.5 cps. In

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