myrmecin (Ia), m.p. 58–59°, which contained 71.42% C and 9.67% H (calc'd 71.39 and 9.59). The infrared spectrum of Ia was found to be identical with that of authentic isoiridomyrmecin and there was no depression in melting point on admixture of Ia from the two sources.

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Synthesis of 11β-Acetoxytestosterone Acetate

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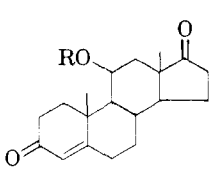
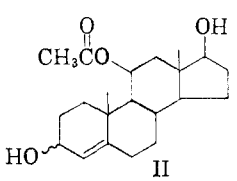
The disappearance of the androgenic and anabolic properties of testosterone upon 11β-hydroxylation[1] led us to examine the effect of esterification of this group. In the case of progesterone, for example, 17α-hydroxylation results in physiological deactivation, whereas the corresponding esters retain activity.[2]

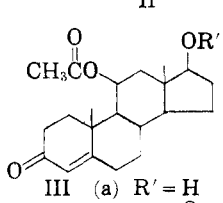
Attempts to prepare 11β-acetoxytestosterone acetate directly from the parent compound[3] were unsuccessful and an indirect route was chosen. Acetylation of 11β-hydroxy-Δ⁴-androstene-3,17-dione[4] (Ia) with special care to reverse the formation of enol acetates, gave the corresponding acetate (Ib). An attempt to perform a selective reduction at C-17 by the method of Norymberski and Woods[5]



I (a) R = H
(b) R = —OCCH₃



II



III (a) R′ = H
(b) R′ = OCCH₃

failed, as did an attempted reduction with yeast.[6] Reduction with sodium borohydride gave instead the triol acetate II, which crystallized as the hemihydrate. Subsequent oxidation of the allylic hydroxyl group with MnO_2[7] gave the desired 11β-acetoxytestosterone (IIIa), which was isolated as the diacetate (IIIb).

In the *levator ani* and seminal vesicle response of castrated rats, compounds Ib, II, and IIIb were essentially inactive.

EXPERIMENTAL[8]

11β-Acetoxy-Δ⁴-androstene-3,17-dione (Ib). 11β-Hydroxy-Δ⁴-androstene-3,17-dione (Ia) (2.00 g.) was suspended in a mixture of 20 ml. of glacial acetic acid and 9 ml. of acetic anhydride, and 200 mg. of *p*-toluenesulfonic acid was added. The resulting suspension was allowed to stand overnight. When almost all of the suspended material had dissolved, the small amount of solid remaining was removed by filtration and the solution was poured slowly onto a slurry of ice. Enough sodium carbonate solution was then added slowly with stirring to adjust to a *p*H of 8 (1.5 hr.) and the oily suspension was stirred for an hour longer. Extraction with ether, followed by washing of the extract with water, drying, and concentration under vacuum, gave a crude solid which was chromatographed on alkaline alumina. The material was placed on the column in benzene and eluted over a broad region (benzene-ether to ether–methylene chloride), combined and crystallized from methylene chloride–ether. There was obtained 1.01 g. of Ib, m.p. 193–194°; $[\alpha]_D$ +179° (diox.); λ_{max}^{MeOH} at 239 mμ (ε = 16,000); IR peaks at 5.78, 6.00, and 6.20μ.

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.22%; H, 8.19%; Found: C, 73.26%; H, 8.10%.

11β-Acetoxy-Δ⁴-androstene-3ξ,17β-diol (II). The acetate Ib (1.00 g.) was dissolved in 2 l. of methanol and cooled to 0°. Sodium borohydride (171 mg.) was added, and the solution was allowed to stand 1 hr. at ice temperature. Excess reagent was destroyed with acetic acid and the solution was evaporated to dryness *in vacuo*. The residue was distributed between water and chloroform, and the organic phase was again evaporated to dryness. This latter residue was then chromatographed on neutral alumina (Woelm) by the gradient elution technique, using benzene and 50% benzene–ethyl acetate solutions as the nonpolar and polar eluants, respectively. From the less polar eluates, 403 mg. of II were isolated and crystallized from moist ether,[9] m.p. 107–112°; no selective absorption in the ultraviolet; $[\alpha]_D^{25}$ +70.5°; IR bands at 2.91, 3.01, 3.20, 5.78, 6.02, and 8.00μ.

Anal. Calcd. for $C_{21}H_{32}O_4 \cdot {}^1/_2H_2O$: C, 70.55%; H, 9.34%; Found: C, 70.27%; H, 9.18%.

From the more polar eluates, a second material was isolated and crystallized from ether to give 30 mg., having m.p. 168–172°. It was not further investigated.

11β-Acetoxylestosterone acetate (IIIb). The triol monoacetate II (600 mg.) was ground to a fine powder, largely

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(8) All melting points were taken on a Kofler Block. Rotations were carried out in a 1-dm. tube at a concentration of *ca.* 1%. Analyses and optical data were obtained by the Microanalytical and Physical Chemistry Departments of these laboratories.
(9) A few droplets of water actually had to be added to the ether to induce crystallization.

dissolved in 6 ml. of chloroform, and agitated overnight with 1 g. of freshly prepared manganese dioxide. The suspension was diluted with warm chloroform, filtered, washed, and concentrated to dryness. The resulting oil had a strong selective absorption around 240 mμ, but could not be crystallized even after chromatography. Acetylation in the usual manner, however, gave 127 mg. of IIIb, crystallized from hexane, m.p. 144–147°; λ_{max}^{MeOH} at 237 mμ (ϵ = 15,900); $[\alpha]_D^{25}$ +117.8; IR peaks at 5.80, 6.01, 6.18, and 8.08.

Anal. Calcd. for $C_{29}H_{32}O_5$: C, 71.10%; H, 8.30%; Found: C, 71.37%; H, 8.32%.

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Occurrence of Scopoletin in the Genus *Brunfelsia*

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Several *Brunfelsia* species (fam. *Solanaceae*) are widely used as ornamental or medicinal plants in Brazil and other South American countries and the roots of *Brunfelsia Hopeana* (Hook.) Benth. (popular name "Manacá") are listed in the Brazilian Pharmacopoeia. The older literature mentions a number of components but none seems to have been satisfactorily characterized so far. Lascelle-Scott[2] mentions the presence of an alkaloid which he named "francisceine." Lenardson[3] also reported on the alkaloid which he named "manacine." The most extensive paper is by Brandl[4] who worked on manacine and its degradation products. Later Peckolt[5] claimed to have isolated still another alkaloid which he named "brunfelsine." Although analytical data are presented by Brandl, none of the mentioned substances had been obtained in a crystalline state. The only crystalline compound mentioned thus far by Lenardson and Brandl is one which was notable because of its very strong blue fluorescence. Brandl[4] believed it to be aesculetin (6,7-dihydroxycoumarin) based on a color reaction and a combustion analysis.

In our own investigations concerning the possible occurrence of alkaloids in the mentioned plant, we could isolate the same fluorescent compound which, however, was not aesculetin but its methyl ether scopoletin, having been identified by mixture melting point and infrared and ultraviolet spectral comparison with an authentic sample.[6] Scopoletin (6-methoxy-7-hydroxycoumarin) has been found to occur in a number of plants of the *Solanaceae* and other families.[7]

Further investigation showed this compound to be present in other *Brunfelsia* species as well. It could be isolated from the following, all collected in the vicinity of Rio de Janeiro:

Brunfelsia Hopeana (Hook.) Benth. (Fraciscea uniflora Pohl.).

Brunfelsia calycina Benth. var. *macrantha* Bailey (Br. grandiflora Don.).

Brunfelsia ramosissima (Pohl.) Benth.

It was also found that the presence of the substance is not limited to the roots, but is general throughout the plants, in roots, stems, twigs, leaves, and flowers. Its remarkable fluorescence allows it to be easily detected hystologically by ultraviolet microscopy.

Isolation of scopoletin was accomplished by first extracting the ground plant material with water and then the aqueous extract continually with chloroform. The crude substance which remained after evaporation of the solvent was then purified by alternate sublimation *in vacuo* and recrystallization from ethanol. The melting point was 204°, the yield in all three species being close to 0.1%.

It is interesting to record that Lenardson, the earliest of the above mentioned investigators, thought that the crystalline compound could possibly be identical with the then recently discovered "gelseminic acid." We know today that "gelseminic acid" is, in fact, scopoletin and Lenardson's assumption was therefore quite correct.

Scopoletin has been found normally to occur only in trace amounts in plants of the family *Solanaceae* (tobacco, potato), but its concentration in the tissues increases significantly as a result of virus infection.[8,9,10] The fact that it is present in relatively high amounts in healthy plants of other genera of the same family suggests a similarity between the metabolism of healthy individuals of one genus with that of diseased individuals of another.

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