Phytochemistry, 1973 Vol. 12, pp. 903 to 906. Pergamon Press. Printed in England.

6β-HYDROXY-4-STIGMASTEN-3-ONE AND 6β-HYDROXY-4-CAMPESTEN-3-ONE

M. G. NAIR* and F. C. CHANG

Department of Molecular and Quantum Biology, University of Tennessee Medical Units, Memphis, Tennessee, TN 38103, U.S.A.

(*Received* 7 *August* 1972. *Accepted* 13 *November* 1972)

Key Word Index—*Melia azedarach*; Meliaceae; 6β-hydroxy-Δ⁴-3-oxo steroids; 6β-hydroxylation.

Abstract—The first naturally occurring 6-hydroxylated Δ⁴-3-oxo steroids with intact sterol side chains have been isolated as a molecular complex from the bark extracts of *Melia azedarach* L. The complex has been characterized by UV, IR, NMR and MS analyses to consist of 6β-hydroxy-4-stigmasten-3-one and 6β-hydroxy-4-campesten-3-one, and these structures confirmed by partial synthesis.

INTRODUCTION

ALTHOUGH numerous steroidal Δ⁴-3-ketones hydroxylated at C-6 have been isolated as metabolites in both *in vivo* and *in vitro* mammalian systems,[1,2] no compound of such type having an intact sterol side chain has been reported as a natural product either from animal or plant sources. We report the isolation and characterization of 6β-hydroxy-4-stigmasten-3-one (Ia) and its close companion, 6β-hydroxy-4-campesten-3-one (Ib), from *Melia azedarach* L.

RESULTS AND DISCUSSION

From the light petrol. extracts of this plant the tetracyclic triterpenoids, kulinone, kulactone, kulolactone and methyl kulonate have been reported previously.[3] By repeated column chromatography of the polar fractions from which methyl kulonate had been removed, we obtained a crystalline material which appeared homogeneous by TLC in several solvents, but which GLC resolved into two components in the proportion of *ca.* 3:1. However, by repeated crystallization, product I was obtained which consisted of 89% (8:1) of the major component, and further recrystallization effected no appreciable change.

Spectrometric studies of I, mp. 212–214°, provided evidence for its characterization: a λ_{max} (EtOH) at 239 nm (ε 13 300), and absorption at 1670 cm^{-1} in the IR, indicated the presence of an α,β-unsaturated ketone group. Additionally in the latter spectrum, a band at 3600 cm^{-1} suggested hydroxyl absorption. High resolution MS determination gave a molecular ion value of 428·3651, corresponding to a molecular formula of $C_{29}H_{48}O_2$, and supporting a hydroxy ketone structure.

* Present address: Nutrition Program, University of Alabama Medical Center, Birmingham, Alabama, AL 35233, U.S.A.

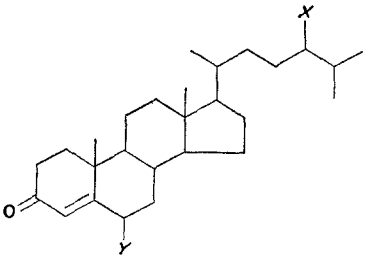
[1] DORFMAN, R. I. and UNGER, F. (1965) *Metabolism of Steroid Hormones*, pp. 382, Academic Press, New York.

[2] CHARNEY, W. and HERZOG, H. L. (1967) *Microbial Transformations of Steroids*, pp. 26, 217, Academic Press, New York.

[3] CHANG, F. C. and CHIANG, C. (1968) *Chem. Commun.* 1156; *idem* (1969). *Tetrahedron Letters* 891; *idem.* (1970) *Lloydia* 33, 491; a full paper is in preparation.

The NMR spectrum of I exhibits typical steroidal methyl resonances in addition to the characteristic and well-documented[4] chemical shift, shape and half-width of both the C-4 olefinic and C-6 (equatorial) protons of a 6β-hydroxy-Δ^4-3-ketone structure. Furthermore, in comparison with the spectrum of 4-stigmasten-3-one (Ic),[5] the effect of 6β-hydroxyl on the chemical shift of the angular methyl groups (C-18 and C-19), as calculated according to Zürcher's correlations,[6] was found to agree well with observed values; the C-18 methyl protons are shifted only slightly (2·5 Hz) downfield, but the C-19 methyl protons because of their 1,3-diaxial juxtaposition with the 6-hydroxy group show a large downfield shift (10·5 Hz).

Thus the major component of I was deduced to be 6β-hydroxy-4-stigmasten-3-one (Ia); and since we previously[7] had isolated from the same bark extracts the C_{29}-compounds, sitosterol and 4-stigmasten-3-one, and each of these was accompanied by its C_{28}-analog, not separable from it by TLC but resolvable by GLC,[8] we speculated that the minor component was 6β-hydroxy-4-campesten-3-one (Ib).



	X	Y	Compound
(Ia)	C_2H_5	OH	6β-Hydroxy-4-stigmasten-3-one
(Ib)	Me	OH	6β-Hydroxy-4-campesten-3-one
(Ic)	C_2H_5	H	4-Stigmasten-3-one

These assignments were confirmed by synthesis: commercially available sitosterol composed mainly of sitosterol and campesterol (*ca.* 3:2 by GLC) was oxidized to a mixture of 6β-hydroxy-4-stigmasten-3-one (Ia) and 6β-hydroxy-4-campesten-3-one (Ib) by a method developed for the preparation of the cholestenone analog.[9] After column chromatography and crystallization, a crystalline material, m.p. 212–214°, $[\alpha]_D$ +24°, was obtained which was identical with I according to m.m.p., IR, UV and NMR comparisons. The MS of I and of the synthetic product II were essentially identical, differing only in relative intensity of fragments that are associated with the different proportion of C_{29}- to C_{28}-analog in the two preparations.

The acetates of I and II were both amorphous, each unresolved by TLC but resolvable by GLC into two components having essentially the same proportion as the original hydroxy

[4] BHACCA, N. S. and WILLIAMS, D. H. (1964) *Applications of NMR Spectroscopy in Organic Chemistry*, pp. 83, 109, Holden-Day, San Francisco; SMITH, L. L. (1964) *Steroids* **4**, 395; TORI, K. and KONDO, E. (1964) *Steroids* **4**, 713; JACKMAN, L. M. and STERNHELL, S. (1969) *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd Edn, pp. 244, 316, Pergamon Press, Oxford.

[5] HAGASHI, S., OKUDE, T., SHIMIZU, A. and MATSUURA, T. (1969) *Chem. Pharm. Bull.* **17**, 163.

[6] ZÜRCHER, R. F. (1963) *Helv. Chim. Acta* **46**, 2054.

[7] To be reported elsewhere.

[8] The ratio of C_{29}- to C_{28}- in each case, sitosterol–campesterol and stigmastenone–campestenone, was also approximately 3:1.

[9] FIESER, L. F. (1953) *J. Am. Chem. Soc.* **75**, 4377.

mixture. The two acetates were identical by IR, UV and NMR.[4] The observation that enrichment of the major (C_{29}) analog in the original isolated mixture from 3:1 to the final 8:1 ratio by fractional recrystallization succeeds, whereas no change was achieved by similar recrystallization of the 3:2 synthetic mixture, suggests that stable 8:1 and 3:2 molecular complexes of the analogs can exist.

TABLE 1. RR_fS AND R_fS OF *Melia* STEROIDS

GLC-3% OV-1 on Gaschrom Q, 2 mm × 183 cm glass column, column temp. 265°

RR_fS	
Compound Ib (C_{28}) 1·00 (5·25′)	Ib acetate 1·11
Compound Ia (C_{29}) 1·22	Ia acetate 1·38

TLC-0·25 mm Silica gel G (Brinkman) plates

Solvent	R_fS Mixture I	R_fS I acetate
Light petrol.–EtOAc (1:1)	0·44	0·67
CH_2Cl_2–EtOAc (9:1)	0·14	0·48
$CHCl_3$–EtOAc (19:1)	0·13	0·56

Plant steroids hydroxylated at C-6 are rare.[10] Our 6-hydroxy compounds presumably are formed in the plant by an enzymatic process,[2] but this is uncertain because Δ^4-3-keto steroids can be hydroxylated (via their dienol ethers) by autooxidation.[11] However, the discovery of the insect-moulting activity of several C_{28}- and C_{29}-phytoecdysones, steroids oxygenated at C-6, lends interest to any potential biogenetic precursors[12] of these compounds.

TABLE 2. HIGH RESOLUTION MS OF I AND II

Mass	Mass to charge ratio (*m/e*)*†	
M^+	428·3655 ($C_{29}H_{48}O_2$)	414·3583 ($C_{28}H_{46}O_2$)
M^+-15	413·3512 ($C_{28}H_{45}O_2$)	399·3340 ($C_{27}H_{43}O_2$)
M^+-18	410·3617 ($C_{29}H_{46}O$)	396·3387 ($C_{28}H_{44}O$)
M^+-side chain	287·2011 ($C_{19}H_{27}O_2$)	
M^+-side chain-18	269·1913 ($C_{19}H_{25}O$)	
M^+-side chain-42	245·1555 ($C_{16}H_{21}O_2$)	
M^+-side chain-42–18	227·1433 ($C_{16}H_{19}O$)	
M^+-side chain-42–93	152·0887 ($C_9H_{12}O_2$)	

* Formulae in parentheses are from high resolution formula tables.

† The first three rows are corresponding *m/e* values from 6β-hydroxy-4-stigmasten-3-one (left) and 6β-hydroxy-4-campesten-3-one.

[10] The only known 6-hydroxy sterols from plant sources: (1) peniocerol, C_{27}, from *Wilcoxia viperinia* [DJERASSI, C., MURRAY, D. H. and VILLOTI, R. (1961) *Proc. Chem. Soc.* 450], (2) macdougallin, C_{28} (C-14 methyl), from *Peniocereus macdougalli* [DJERASSI, C., KNIGHT, J. C. and WILKINSON, D. J., (1963) *J. Am. Chem. Soc.* **85**, 835], and (3) stigmastan-3β,5α,6β-triol, C_{29}, recently reported as a component of sugar cane wax [DESHMANE, S. S. and DEV, S. (1971) *Tetrahedron* **27**, 1109].

[11] GARDI, R. and LUSIGNANI, A. (1967) *J. Org. Chem.* **32**, 2647.

[12] HEFTMANN, E. (1970) *Steroid Biochemistry*, p. 24, Academic Press, New York.

EXPERIMENTAL

NMR spectra were taken in $CDCl_3$ with TMS as internal standard; chemical shifts are expressed in τ units. Column and TLC were performed with Merck silica gel H. GLC was carried out on 3% OV-1 on Gaschrom Q at column temp. of 270° on a Hewlett-Packard 5750 instrument equipped with hydrogen flame detector and disc integrator. High resolutions MS were performed on an AEI MS-902 instrument with computer processing.

6β-Hydroxy-4-stigmasten-3-one–6β-hydroxy-4-campesten-3-one by isolation (8:1 mixture), I. In the more polar fractions of the column chromatography of petroleum ether extracts of *Melia azedarach* L.,[3] which contained methyl kulonate as the major component, a minor product was detected by TLC. After further column chromatography of these fractions, most of the methyl kulonate was removed by crystallization. The mother liquor residue which contains the minor component (estimated yield, 0·003% of the powdered dried bark) was chromatographed to obtain crystalline material which was homogeneous by TLC [light petrol.–EtOAc (1:1), or EtOAc–$CHCl_3$ (10:1), or $CHCl_3$]. By GLC, however (Table 1) the material was resolved into two components in the ratio of *ca.* 3:1. Continued fractional crystallization from MeOH afforded an increase in the proportion of the major component until 89% purity was reached; further crystallization effected no additional enrichment. Product I was colorless crystals, M^+ 428·3651, m.p. 212–214°; $[\alpha]_D$ +24°; λ_{max} (EtOH) 239 nm (ϵ 13 300); λ_{max} ($CHCl_3$) 3600, 1670 cm^{-1}; NMR: 3H *s* at τ 9·25 and 8·62, 1H *t* centered at τ 5·80 ($W_{1/2}$ 7 Hz), 1H *s* at τ 4·18.

6β-Hydroxy-4-stigmasten-3-one–6β-hydroxy-4-campesten-3-one by partial synthesis (3:2 mixture), II. Commercial sitosterol [K & K Co. product, essentially a mixture of sitosterol and campesterol (ratio, 3:2) according to GLC] was oxidized by the sodium dichromate method of Fieser[9] for the 6β-hydroxylation of 4-cholesten-3-one. After processing, the main product of the reaction separating from light petrol. was found to be homogeneous by TLC, but by GLC consisted of two components in the ratio of 3:2. Recrystallization from MeOH did not change this ratio. The colorless crystals, characterized as a 3:2 mixture of 6β-hydroxy-4-stigmasten-3-one and 6β-hydroxy-4-campesten-3-one, melted at 212–214°; $[\alpha]_D$ ($CHCl_3$) +24°; λ_{max} (EtOH) 239 nm (ϵ 13 200); λ_{max} ($CHCl_3$) 3600, 1670 cm^{-1}; NMR: 3H-*s* at τ 9·25 (C-18 Me) and 8·62 (C-19 Me); 1H-perturbed *t* at τ 5·80 (C-6H), 1H-slightly broadened *s* at τ 4·18 (C-4H). (Found: C, 81·2; H, 11·1. $C_{29}H_{48}O_2$–$C_{28}H_{46}O_2$ (3:2) requires: C, 81·2; H, 11·2.) I and II are identical according to comparisons of their optical rotation, UV and IR spectra, and by m.m.p. Their NMR spectra exhibit identical prominent signals, differing very slightly in the intensity of secondary signals which can be attributed to the difference in proportion of the analogs. The differences in intensity of certain ions in the MS are explained in the same way.

Acetates of I and II. The acetates of both were prepared at room temp. with Ac_2O in pyridine. Both products were amorphous and as with the parent alcohols were homogeneous by TLC but resolvable by GLC into two components, in the original ratios; 8:1 for I and 3:2 for II. The acetate of I had λ_{max} (EtOH) 239 nm (ϵ 13 300); in the IR had no absorption in the OH stretching region, but had absorption at 1727 (ester carbonyl) and 1670 (α,β-unsaturated ketone) cm^{-1}, and its NMR spectrum showed 3H-*s* at τ 9·23 (C-18 Me), 8·70 (C-19 Me) and 7·95 ($-OOCCH_3$); 1H-perturbed *t* centered at τ 4·56 (C-6H); 1H-slightly broadened *s* τ 4·05 (C-4H). The acetates of I and II had identical UV and IR spectra, and nearly identical NMR spectra, differing only very slightly in the background or methylene envelope.

High resolution MS of I and II. The MS of I and II were nearly identical, differing only in the relative intensities of the peaks associated with the side chain (see top three rows in Table 2), reflecting the ratios of C_{29} to C_{28} analog in the two mixtures.

Acknowledgements—This work was supported in part by U.S. Public Health Service Grants, CA-11507 and CA-05011, from the National Cancer Institute. We are indebted to the Research Triangle Center for MS, through the kindness of Dr. David Rosenthal, for high resolution MS assistance.