Phytochemistry, Vol. 21, No. 7, pp. 1814–1816, 1982.
Printed in Great Britain.

0031–9422/82/071814–03$03.00/0

ISOLATION OF DIHYDROXYLUPENE AND DIHYDROXYLUPANE FROM THE BARK OF *LAWSONIA INERMIS**

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(*Received* 20 *October* 1981)

Key Word Index—*Lawsonia inermis*; Lythraceae; henna; triterpenoids; 30-hydroxylupeol; 30-hydroxylupanol.

Abstract—Two pentacyclic triterpenes isolated from the bark of henna were identified as 3β, 30-dihydroxylup-20(29)-ene (hennadiol), and (20*S*)-3β, 30-dihydroxylupane. The assignment of the C-20 configuration in the latter compound was supported by the analysis of Eu(fod)$_3$-induced ^{1}H NMR chemical shifts in the two C-20 epimers synthesized from lupeol.

INTRODUCTION

Previously we have reported the tentative identification of a pentacyclic triterpene hennadiol, isolated from the bark of *Lawsonia inermis* L., syn. *L. alba* L. (Lythraceae), as the derivative of ψ-taraxastene‡ [1]. The same plant was also found to produce four lupane derivatives, lupeol, 3β-hydroxy-20-oxo-30-nor-lupane, betulin, and betulinic acid [2]. Further investigation of hennadiol, described here, established that this compound is also a lupane derivative and revealed the presence of the corresponding dihydro derivative.

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of hennadiol measured in pyridine-d_5–$CDCl_3$ displayed only six angular methyl group singlets at δ 1.12, 1.03, 0.96, 0.86, 0.80, and the set of broad singlets at 5.22 (1 H), 4.99 (1 H), and 4.24 (2 H) along with the characteristic 3α-H double doublet at 3.29 and two broad OH signals at 5.39 and 4.62. The acetylation confirmed the presence of two hydroxyl groups and the spectrum of hennadiol diacetate showed two OAc singlets at 2.10 and 2.05, a 3α-H signal at 4.45, a two proton singlet at 4.54, and two olefinic proton signals at 4.93 and 4.89. These NMR data conformed with the presence of the fragment CH_2=C–CH_2–OR and pointed to the most likely structure of hennadiol as 30-hydroxylupeol, 3β, 30-dihydroxylup-20(29)-ene, **1e**. This structure was corroborated by mass spectra of hennadiol and its diacetate which were particularly informative when measured below 20 eV.

The reference compound **1d**, identical with hennadiol diacetate, was prepared from lupeol acetate, **1a**, by selenium dioxide oxidation [4] to the α, β-unsaturated aldehyde **1b**, subsequent reduction with sodium borohydride to the allylic alcohol **1c**, which was formed as the sole product, and acetylation. 30-Hydroxylupeol was recently isolated from several plant sources [5–8].

The ^{1}H NMR spectrum of the crude hennadiol showed the additional signal of a doublet at 3.51, J = 6.9 Hz. This signal indicated the presence of the second related component which was separated as a diacetate by argentation chromatography. Its ^{1}H NMR spectrum showed only two signals in the medium-field part, the characteristic 3α-H double doublet at 4.46 and two-proton doublet at 3.83, J = 7.0 Hz. The mass spectrum of this diacetate also conformed with the structure of the saturated pentacyclic triterpene diol diacetate and pointed to one of the two C-20 stereoisomers of 3β, 30-diacetoxylupane as the most probable alternative.

In order to prepare these reference compounds, the α,β-unsaturated aldehyde **1b** was exhaustively hydrogenated to the mixture of the two monoacetates **2a** and **3a**. The ^{1}H NMR spectrum of this mixture showed two sets of CH–CH_2–OH signals, the doublet at 3.41 (J = 6.9 Hz), and the AB pattern, the part of an ABX system at 3.83 (*dd*, J = 10.0, and 4.4 Hz), and 3.99 (*dd*, J = 10.0, and 7.5 Hz). The observation of the formation of both C-20 stereoisomers, in the ratio close to 1:1, as a result of the hydrogenation of **1b** contradicted earlier reports of the isolation of only one product [7, 9, 10]. The separation of these two monoacetates, although difficult, was finally accomplished by HPLC. The stereochemical differentiation of these C-20 epimers was discussed previously based

*Part 5 in the series "Constituents of Indian Medicinal Plants". For Part 4, see Pyrek, J. St., Kocor, M., Sharma, B. R. and Atal, C. K. (1977) *Rocz. Chem.* **51**, 1679.

‡Traditional names of pentacyclic triterpene hydrocarbons taraxastene, ψ-taraxastene and taraxastane are misleading and suggest their relation with sterols. In order to avoid this confusion the following abbreviations were proposed: taraxene, ψ-taraxene and taraxane [3].

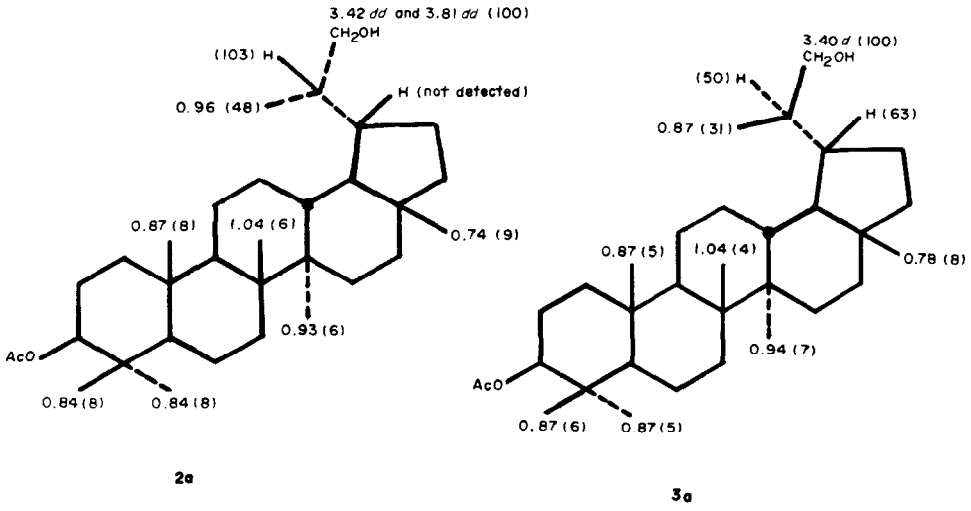

Fig. 1. ^{1}H NMR chemical shifts and relative Eu(fod)$_3$-induced shifts of two 20-epimeric 3β-acetoxy-30-hydroxylupanes.

solely on the analogies with 28,30-dihydroxylated lupanes [12]. An alternative, direct confirmation is described below.

The analysis of space-filling models indicated that the rotamer with 20-H facing toward ring C predominates, as the steric hindrance of the side-chain is primarily due to the 12-methylene group. Thus the hydroxymethylene group locates above the plane for the 20*S* epimer, **3a**, and below the plane for the 20*R* epimer, **2a**. Moreover, in the case of the 20*R* epimer the restricted rotation of the 20–30 C–C bond may be responsible for the pronounced non-equivalence of C-30 protons. In the same 20*R* epimer, the signal of Me-28 is expected to be upfield, whereas the signal of Me-29 is expected to be downfield when compared to those of the 20*S* epimer. In the straightforward manner, the different disposition of the 30-OH group with respect to the skeleton was demonstrated by europium-induced shifts measured for the two monoacetates **2a** and **3a** and normalized assuming $\Delta\delta = 100$ for 30-H$_2$ (Fig. 1) [13]. A dramatic difference was observed for the 19β-H signal shifted downfield only in the case of one isomer and was identified by double resonance. Therefore, this isomer was unequivocally assigned the configuration 20*S* and the structure **3a**. Only in the case of **3a** does the 30-OH group reside in the close vicinity of 19β-H and allow for appreciable interaction. This assignment conforms with the ^{1}H NMR differences discussed above and confirms that obtained before [11]. The diacetate **5b**, obtained from the more polar monoacetate **3a** was entirely identical with the diacetate obtained from the natural diol isolated from *Lawsonia*.

EXPERIMENTAL

Isolation of hennadiol. The plant was collected in Calcutta and the identification was confirmed by Dr P. C. Dutta, Reader, Department of Botany, University of Calcutta. Air-dried and powdered bark (2.5 kg) was extracted with petrol for 30 hr. The extract was concentrated and kept at 0° for 2 days. This resulted in the deposition of the colorless, insoluble residue (1 g). The mother liquor was chromatographed on neutral alumina to give a mixture of triterpenoids (0.5 g, R_f 0.45, 0.49, and 0.52, CHCl$_3$–MeOH, 98:2) eluted with C$_6$H$_6$–CHCl$_3$.

This fraction was rechromatographed on Si gel with C$_6$H$_6$–CHCl$_3$, 1:2, to give crude hennadiol (65 mg, single spot R_f 0.49), mp 230–232°, $[\alpha]_D = -13°$, 5% in CHCl$_3$. It was acetylated (28 mg, Ac$_2$O–C$_5$H$_5$N room temp.) and separated by prep. TLC on Si gel–AgNO$_3$ with three successive developments in C$_6$H$_6$.

Hennadiol diacetate, **1d**. The more polar compound from the above separation was recrystallized from MeOH (16 mg), mp 164–167°, not depressed by admixture with synthetic **1d** and identical by MS and ^{1}H NMR comparison.

30-Hydroxylupanol diacetate, **3d**. The less polar compound was recrystallized from MeOH (8.5 mg), mp 226–229°, not depressed by admixture with **3d** and identical by spectral comparison. ^{1}H NMR (100 MHz, CDCl$_3$): δ 4.46 (*dd*, 3α-H), 3.83 (*d*, $J = 7.0$ Hz, 30-H$_2$), Me: 2.05 (*s*, 2 × Ac), 1.04 (*s*, 26), 0.93 (*s*, 27) 0.86 (*s*, 23, 24, 25), 0.82 (*d*, $J = 7$, 29 Hz), and 0.77 (*s*, 28); EIMS (probe) 15 eV, *m/z* (rel. int.): 528 (M$^+$) (3), 468 (M–AcOH) (100), 453 (M–AcOH–Me) (21), 425 (M–AcOH–43) (20), 408 (M − 2 × AcOH) (5), 386 (ABCD ion) (14), 249 (AB ion) (4), 190 (30), 189 (AB ion) (40).

3β-Acetoxy-30-oxolup-20(29)-ene, **1a**. Lupeol acetate, **1a** (201 mg) in HOAc (15 ml) was refluxed for 2 hr with SeO$_2$ (400 mg). The less polar product was separated by prep. TLC (Si gel, C$_6$H$_6$–EtOAc, 19:1), mp 225°, lit. [4] 226; λ_{max}^{EtOH} 225 nm (log $\epsilon = 3.7$); ν_{max}^{KBr} cm^{-1}: 3050, 2670, 1720, 1620, 1240; ^{1}H NMR (100 MHz, CDCl$_3$): δ 9.49 (*s*, 30-H), 6.28 and 5.91 (singlets, 29-H$_2$), 4.46 (*dd*, 3α-H), 2.74 (*m*, 19-H), Me: 2.02 (*s*, Ac), 1.01 (*s*, 26), 0.92 (*s*, 27), 0.84 (*s*, 23, 24, 25), and 0.82 (*s*, 28).

3β, 30-Diacetoxylup-20(29)-ene, **1d**. The above α, β-unsaturated aldehyde, **1b**, was reduced with NaBH$_4$ in MeOH to give 3β-acetoxy-30-hydroxylup-20(29)-ene, **1c**, as the sole product; ^{1}H NMR (100 MHz, CDCl$_3$): δ 4.90 (*br*, 29-H$_2$), 4.46 (*dd*, 3α-H), 4.10 (*b*, 30-H$_2$), Me: 2.03 (*s*, Ac), 1.03 (*s*, 26), 0.94 (*s*, 27), 0.84 (*s*, 23, 24, 25), and 0.78 (*s*, 28). It was acetylated at room temp. to give **1d** recrystallized from MeOH, mp 165–166, lit. [4] 165–166°; ^{1}H NMR (100 MHz, CDCl$_3$): δ 4.94 (*br s*, 29-H), 4.91 (*br s*, 29-H), Me: 2.09 (*s*, Ac), 2.03 (*s*, Ac), 1.03 (*s*, 26), 0.95 (*s*, 27), 0.86 (*s*, 23, 24, 25), and

0.79 (*s*, 28); EIMS (probe) 15 eV, *m/z* (rel. int.): 526 (M^+) (31), 511 (M–Me) (2), 484 (M – ketene) (7), 466 (M–AcOH) (24), 451 (M–AcOH–Me) (9), 423 (M–AcOH–43), 406 (M – 2 × AcOH) (4), 357 (4), 297 (5), 249 (AB ion) (7), 216 (12), 201–203 (20), 189 (AB ion) (50), 43 (100). The alkaline hydrolysis produced 3β,30-dihydroxylup-20(29)-ene, **1e**, identical with natural hennadiol, mp 236°; EIMS (probe) 70 eV, *m/z* (rel. int.): 442 (M^+) (14), 424 ($M-H_2O$) (16), 409 ($M-H_2O$–Me) (11), 384 (M – side-chain-H) (26), 381 ($M-H_2O-43$) (13), 207 (68), 189 (97), 81 (100).

(20R)- and (20S)-3β-*Acetoxy*-30-*hydroxylupane*, **2a** *and* **3a**. α, β-Unsaturated aldehyde, **1b** (50 mg) in AcOH (5 ml) was reduced over Adams catalyst (50 mg) for 20 hr. Main products were separated by HPLC (Si gel, four columns, 30 × 0.8 cm, in series, hexane–EtOAc, refractive index detector). Less polar monoacetate, **2a**: ^{1}H NMR (100 MHz, $CDCl_3$): δ 4.48 (*dd*, 3α-H), 3.81 (*dd*, 30-H_a, J_{ab} = 10 Hz, $J_{a,20}$ = 4.4 Hz), 3.42 (*dd*, 30-H_b, $J_{b,20}$ = 7.0 Hz), Me: 2.04 (*s*, Ac), 1.04 (*s*, 26), 0.96 (*d*, 29, *J* = 7 Hz), 0.93 (*s*, 27), 0.87 (*s*, 25), 0.84 (*s*, 23, 24), and 0.74 (*s*, 28). It was acetylated to give (20*R*)-3β,30-diacetoxylupane, **2b**, recrystallized from MeOH (12 mg), mp 160–165, lit. [9] 163–164°, lit. [11] mp 160.

More polar monoacetate, **3a**: ^{1}H NMR (as above): δ 4.47 (*dd*, 3α-H), 3.40 (*d*, 30-H_2, *J* = 6.9 Hz), Me: 2.04 (*s*, Ac), 1.04 (*s*, 26), 0.94 (*s*, 27), 0.88–0.86 (*br s*, 23, 24, 25, 29), and 0.78 (*s*, 28). It was acetylated to give (20*S*)-3β, 30-diacetoxylupane (**3b**) recrystallized from MeOH (15 mg), mp 225°, lit. [11] 218–220°.

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Phytochemistry, Vol. 21, No. 7, pp. 1816–1818, 1982.
Printed in Great Britain.

0031-9422/82/071816-03$03.00/0

10-NONACOSANOL, SITOSTEROL AND NONACOSANEDIOLS IN *JUNIPERUS PINCHOTII*

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(*Received* 18 *September* 1981)

Key Word Index—*Juniperus pinchotii*; Cupressaceae; juniper leaves; non-saponifiables; 10-nonacosanol; sitosterol; nonacosanediols.

Abstract—GC of juniper leaf non-saponifiables gave three peaks of sterol-triterpenes which were identified as 10-nonacosanol, sitosterol and a mixture of nonacosanediols.

INTRODUCTION

A recent, privately published book, *Atlas of Gas Chromatography on Phytosteroids in Japan* [1] shows GC separation diagrams (1% OV-101, 210°) of the non-saponifiable fractions obtained from lipid extracts of the leaves of 1296 plants. The major component of this fraction obtained from numerous plants in the families Pinaceae, Taxodiaceae, Cupressaceae (genus *Chamaecyparis* and especially three species in the genus *Juniperus*), Ranunculaceae, Lardizabalaceae, Berberidaceae, Hamamelidaceae (genus *Corylopsis*), Caprifoliaceae (genus *Lonicera*) and Rosaceae (genus *Sorbus*, *Spiraea*) appeared as a peak with a retention time between cholesterol (*RR*, 1.00) and campesterol (*RR*, 1.30) in the GC diagrams. During a recent trip through Texas (May 1981) leaves