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

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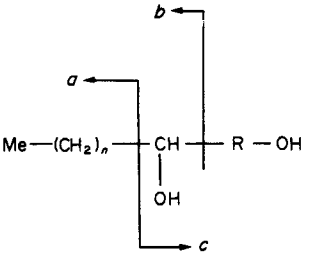
Key Word Index—*Juniperus pinchotii*; Cupressaceae; juniper leaves; non-saponifiables; 10-nonacosanol; sitosterol; nonacosanediols.

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

Table 1. Mass spectral analysis of nonacosanediol mixture



OH on C no. (from end of chain)	Fragment, *m/z* (rel. int.)					
	a	*b*	*b*–H_2O	*c*	*c*–H_2O	*c*–$2H_2O$
4	—	73 (9.5)	55 (99.9)	397 (0)	379 (3.5)	361 (2.4)
5	57 (100)	87 (14)	69 (92.0)	383 (0)	365 (4.2)	347 (4.2)
7	85 (38)	115 (2)	97 (67.5)	355 (0.5)	337 (6.5)	319 (1.4)
10	127 (6.9)	157 (0)	139 (8.1)	313 (0)	295 (5.1)	277 (0.6)
20	267 (0.3)	297 (9.6)	279 (1.3)	173 (11.1)	155 (46.4)	137 (60.5)

of *Juniperus pinchotii* were collected near the Macdonald Observatory (elevation 2300 m) in the Davis Mountains in order to determine the nature of the material with this retention time.

RESULTS AND DISCUSSION

The solvent extract of the leaves was hydrolysed sequentially with acid and alkali. The non-saponifiable fraction gave three peaks on GC, RR_t (cholesterol) 1.17, 1.63 and 2.00 with area ratios of 3:1.6:1.1, respectively. The three components were separated by chromatography and crystallization and identified as 10-nonacosanol (RR_t 1.17), sitosterol (RR_t 1.63) and a mixture of at least three nonacosanediols (RR_t 2.00).

10-Nonacosanol has been shown to be a component of surface waxes of numerous plants [2, 3] and it has been isolated together with sitosterol from three other junipers [4–6]. Nonacosanediols have been isolated from *Juniperus oxycedrus* [6], *Pinus radiata* [7], *Rosa damascena* [8] and *Rhus cotinus* [9]. Comparison of the MS of the present mixture to published values [7–9] suggested it to be composed of nonacosanediols with OH groups in positions 4, 5, 7, 10 and 20 suggesting the presence of 4, 10-, 5, 10-, 7, 10- and 10, 20-diols. The main point to be made here, however, is to draw attention to the fact that 10-nonacosanol and nonacosanediols appear in the regions of GC separation diagrams that are commonly attributed to sterols such as lathosterol, brassicasterol, ergosterol and 5,6-dihydroergosterol ($RR_{t\,chol.}$: 1.11, 1.11, 1.20 and 1.24, respectively) and triterpenes such as parkeol, cycloartenol, butyrospermol, α- and β-amyrin and lupeol ($RR_{t\,chol.}$: 2.00, 2.00, 1.92, 2.10, 1.86 and 2.18, respectively [10]).

EXPERIMENTAL

Freshly collected leaves were extracted continuously with EtOH, EtOH–C_6H_6 and Me_2CO. The extract was hydrolysed with 1 N HCl in EtOH and then saponified, and the non-saponifiable fraction extracted with Et_2O. This orange semi-solid material gave three peaks in the sterol–triterpene region on GC (5% OV-101, 250°) and 14 spots on TLC R_f 0.16–0.80 (Si gel, 6:4 hexane–EtOAc), the majority corresponding to the many lower MW terpenoids in *J. pinchotii* [11].

Crystallization of the non-saponifiable fraction from MeOH at −15° gave a precipitate which when crystallized from MeOH–$CHCl_3$, EtOH, Me_2CO and C_6H_6 gave white needles of the substance of RR_t 1.17, R_f 0.70, mp 81.5–82.5° (corr.). Its MS and NMR [12] suggested the compound to be 10-nonacosanol and its mp and that of its acetate (46–47°, corr., lit. [4] 77–78°, acetate 45–46°, lit. [5] 82°, acetate 49°) confirmed the spectroscopic evidence.

The MeOH mother liquors from the original crystallization were evaporated and the residue crystallized from Me_2CO to give the substance of RR_t 1.63 which was shown to be sitosterol (mmp, GC and TLC [13]).

The residual mother liquors from sitosterol were evaporated and the residue chromatographed on a Si gel column with 20% Et_2O–light petrol (l.p.) followed by 60% Et_2O–l.p. to give the material of RR_t 2.00, R_f 0.40, 0.37. It was crystallized from EtOAc to yield three fractions, mp: (a) 89.5–90°, (b) 90.5–100°, (c) 87–89°, lit. for 5, 10-diol [6] 91–93°. All gave the same three spots on Si gel TLC with 80:20:1 l.p.–Et_2O–HOAc, R_f 0.40, 0.32, 0.28 (7 hr continuous development, upper cm of plate protruded through a slit in the aluminium foil cover of the tank). The MS of fraction (a), *m/z* 422 ($M^+ - H_2O$, 1.3%), 404 ($M^+ - 2H_2O$, 4.6%), gave peaks (Table 1) suggesting the presence of OH on carbon atoms 4, 5, 7, 10 and 20. Absence of a peak at *m/z* 325 precluded the 5, 8-diol [8] as a component of the mixture.

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TWO NEW ISOPRENOID SPIRO COMPOUNDS FROM POTATO TUBERS INFECTED WITH *PHOMA EXIGUA*

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; *Phoma exigua* var. *foveata*; isoprenoids; 6, 10-dimethylspiro [4,5] dec-6-ene-2, 8-dione; 2-(1′, 2′-dihydroxy-1′-methylethyl)-6, 10-dimethyl-9-hydroxy-spiro-[4,5] dec-6-en-8-one.

Abstract—Two new isoprenoid compounds, 6, 10-dimethylspiro [4,5] dec-6-ene-2, 8-dione and 2-(1′, 2′-dihydroxy-1′-methylethyl)-6, 10-dimethyl-9-hydroxyspiro [4,5] dec-6-en-8-one, have been isolated from potato tubers infected with *Phoma exigua* var. *foveata*.

Coumarins and sesquiterpenes in the protective zone produced by potato tubers infected with the fungus *Phoma exigua* var. *foveata* have been the subject of a previous study [1]. Two new isoprenoids, **1** and **2**, from the same source have now been isolated and identified.

In its ^{1}H NMR spectrum, compound **1** showed a pattern for a cyclohexenone ring similar to that of compound **3** reported previously [1]. According to the ^{13}C NMR spectrum it contained, together with the signals from the cyclohexenone ring, an additional carbonyl signal (δ 216.7) and three triplets indicating a cyclopentanone ring. The presence of a cyclopentanone was also confirmed by an additional absorption from a carbonyl group at 1740 cm^{-1} in the IR spectrum. High-resolution mass spectrometry gave the molecular formula $C_{12}H_{16}O_2$. On the basis of these spectral data, compound **1** was given the structure 6, 10-dimethylspiro [4,5] dec-6-ene-2, 8-dione.

Strong similarities were noted between the data for compound **2** and the corresponding data for **3**. Significant differences in the ^{1}H NMR spectrum were noted only for H–9 and Me–10. The H–9 appeared as a doublet at δ 3.83, and the Me–10 was shifted downfield 0.2 ppm compared with the signals in the spectrum of **3**. In the ^{13}C NMR spectrum of **2**, C–9 and C–10 were shifted downfield (δ 31.3 and 8.6, respectively) while Me–10 was shifted upfield (δ 3.6) compared with the corresponding data of **3**. These data, and the mass spectrum showing M^+ at *m/z* 268 (1.7 and 5.1% rel. int. at 70 and 20 eV, respectively) indicated hydroxyl substitution at C–9. The conformation of the cyclohexenone ring in **2** was fixed by the coupling constant (12.7 Hz) between the protons H–9 and H–10, corresponding to an a–a orientation of the two protons [2]. Hence, the structure of compound **2** is 2-(1′,2′-dihydroxy-1′-methylethyl)-6, 10-dimethyl-9-hydroxyspiro-[4, 5]-dec-6-en-