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3α, 16α-DIHYDROXYTARAXENE-3-ACETATE: A NEW TRITERPENE FROM *CENTAUREA SOLSTITIALIS*

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Key Word Index—*Centaurea solstitialis*; Compositae; 3α, 16α-dihydroxytaraxene-3-acetate; taraxastane-type triterpene; *n*-nonacosane; antitumor testing.

INTRODUCTION

Centaurea solstitialis L. has been investigated for the presence of alkaloids [1] and cyanogenic glycosides [2]. The isolation of sesquiterpene lactones from an ethyl acetate extract has aslo been reported [3–5]. Our observation that an alcoholic extract of the plant showed activity versus P-388 lymphoid leukemia in mice prompted a detailed chemical examination in an effort to isolate the active constituent.

RESULTS AND DISCUSSION

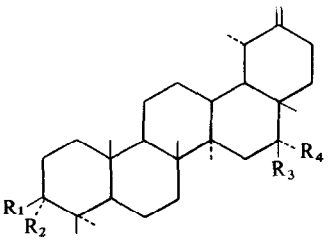
Fractionation of both an alcoholic and ethyl acetate extract showed that the antileukemic activity resided in a light petrol soluble oil. Filtration of this oil in anhydrous ethyl ether through Florisil resulted in a fraction from which *n*-nonacosane (mp, IR, NMR, high resolution MS) precipitated upon treatment with cold acetone. The acetone soluble fraction remaining was chromatographed on Si gel. Elution with chloroform resulted in the isolation of a white solid, recrystallized from acetonitrile or ethanol, yielding pure substance A, mp 224–226°.

PMR and MS analysis of A indicated it was probably the monoacetate of a triterpene diol. The phytochemistry of the Compositae suggested A be based on taraxastane, represented by taraxasterol (**1**), and pseudotaraxasterol (**2**) [6], and the corresponding diols, arnidiol, **3**, and faradiol, **4** [7–10]. Substance A, after hydrolysis, oxidation to the dione and isomerization of the exocyclic double-bond gave faradione (**5**) [7]. Comparison with authentic (**5**) (mmp, TLC, IR) indicated identity thus establishing the carbon skeleton and stereochemistry at all centres excepting C-3 and C-16.

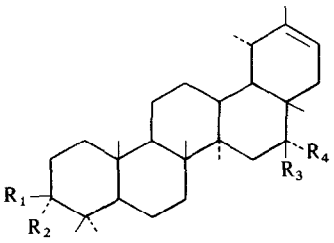
Comparison of the PMR spectrum and MS fragmentation of A with reported and expected values showed close agreement with arnidiol 3-acetate, excepting the downfield positions of the protons adjacent to the oxygen functionalities (found: C-3-H, δ 5.35, J = 2.5 Hz; C-16-H, δ 4.47, J = 3.6 Hz; c.f. arnidiol 3-acetate: C-3-H, δ 4.0–4.8, J = 7.9 Hz; C-16-H, δ 3.3–4.0, J = 5,11 Hz) suggesting an α configuration of both oxygens. Comparison of these coupling constants and chemical shifts with those reported in similar triterpenes and steroids [11], suggested assignment of the C-3-acetate and C-16-hydroxyl to the α (axial) configuration. Substance A is thus formulated as 3α, 16α-dihydroxytaraxene-3-acetate, (**6**), the first known report of a taraxastane with diol axial oxygen configuration. It was inactive in the P-388 tumor system [12].

EXPERIMENTAL

All mps are uncorr. The NMR spectra were recorded in $CDCl_3$, with TMS as int. stand., on a Jeol 100 MHz spectrometer. The powdered above-ground plant was exhaustively extracted with EtOAc to yield a dark tar (4.35%). Trituration



1 R_1 = OH R_2 =, R_3 =, R_4 = H
3 R_1 = R_3 = OH R_2 = R_4 = H
6 R_1 = R_3 = H R_2 = R_4 = OH



2 R_1 = OH R_2 =, R_3 =, R_4 = H
4 R_1 = R_3 = OH R_2 = R_4 = H
5 R_1, R_2 = R_3, R_4 = O

of the tar with light petrol (30–60°) yielded 14% of soluble oil. Filtration of this oil in anhydrous Et_2O through Florisil afforded a yellow oil from which an insoluble solid (22%) and an Me_2CO insoluble oil (78%) were obtained by repetitive treatment with cold (−20–0°) Me_2CO.

n-*Nonacosane*. The Me_2CO insoluble solid crystallized from Me_2CO or EtOH as colorless needles, mp 62–63° (lit. *n*-nonacosane, 62.7–63°). ν_{max}^{KBr} cm^{-1}: 2900, 1450, 1390, 740. δ values: 0.9 (*t*) (6H), 1.2 (*m*) (54H). MS *m/e* 408 (M^+), regularly spaced fragments to 71, 57, 43. High resolution MS: 408.468 ($C_{29}H_{60}$ requires 408.470).

3α, 16α-dihydroxytaraxene-3-acetate (*substance A*). The Me_2CO soluble oil was chromatographed over Si gel. Elution with $CHCl_3$ yielded a crude white solid from which additional *n*-nonacosane was recovered by treatment with cold Me_2CO. The Me_2CO soluble solid (Substance A) remaining was crystallized from CH_3CN or EtOH as colorless needles, mp 224–226°. ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2900, 1740 (MeCO), 1450, 1370, 1240, 1000. δ values: 0.84 (*s*) (12H), 0.92 (*s*) (3H), 1.16 (*d*, J = 6 Hz) (3H), 1.18 (*s*) (3H), 2.04 (*s*) (3H), 4.47 (*m*, J = 3.6 Hz) (1H), 4.61 (*br s*) (2H), 5.35 (*m*, J = 2.5 Hz) (1H). MS: 484 (M^+), 466 ($M-H_2O$), 408, 249, 205, 189. High resolution MS: 466.381 ($C_{32}H_{50}O_2$ requires 466.381).

3α, 16α-dihydroxytaraxene. Substance A hydrolysed at 60° for 1 hr, in MeOH–HCl. Work up in the usual way gave the diol mp 252–255°.

Arnidione. The diol was oxidised with Jones' reagent in Me_2CO at 0° for 5 min. Dilution with H_2O afforded the dione mp 252–254°.

Faradione (**6**). The dione was refluxed for 16 hr in EtOH–C_6H_6–H_2SO_4 (10:5:1). Dilution with H_2O and extraction with Et_2O yielded faradione from EtOH, mp 209–212°. (mmp, IR, TLC).

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CUCURBITACINS IN *PURSHIA TRIDENTATA*

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Key Word Index—*Purshia tridentata*; Rosaceae; triterpenes; cucurbitacins D and I; ^{13}C-NMR; seed germination inhibitor; chemotaxonomy.

INTRODUCTION

Purshia tridentata (Rosaceae, Antelope Bitterbrush) is a widespread native shrub occurring on rangelands of the Western United States [1]. It is important as a large game winter forage plant for both deer and domestic stock [2] as well as a ground cover in range management programs [3].

The presence of a seed germination inhibitor in the seeds of *P. tridentata* has hindered propagation of this species as part of range management programs [4]. The inhibitor appears to be deactivated by treatment of the seeds with thiourea. It is a relatively polar substance and appears to be somewhat soluble in water. The work reported in this paper was undertaken in an attempt to isolate this inhibitor.

RESULTS

The ethyl acetate extracts of the defatted seeds showed the highest inhibitory activity; thus, the inhibitor was a relatively polar material. Extraction of the seeds directly with ethanol or hot water also gave extracts of high inhibitory activity but containing large amounts of phenolic impurities. Initial attempts to fractionate the ethyl acetate extracts by Si gel chromatography resulted in the loss of activity. Column chromatography on polyamide was then used; the active fraction was obtained by elution with acetone. Further work-up by rechromatography and crystallization of the active fraction resulted in the isolation of two related triterpenes.

The PMR spectrum of the major triterpene suggested the presence of eight C-methyl groups. A vinyl AB