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

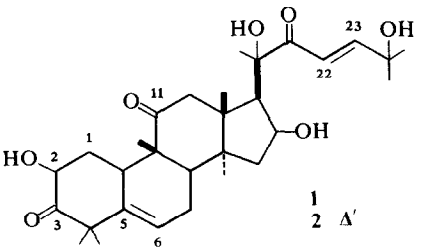
The ethyl acetate extracts of the defatted seeds showed the highest inhibitory activity; thus, the inhibitor was a relatively polar material. Extraction of the seeds directly with ethanol or hot water also gave extracts of high inhibitory activity but containing large amounts of phenolic impurities. 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

doublet was also easily distinguished. The mp, below 200°, suggested that the compound was a tetracyclic rather than a pentacyclic triterpene. The ^{13}C-NMR spectrum indicated the presence of three carbonyl groups, two double bonds and four carbons having single bond oxygen attached. These data suggested a cucurbitacin structure and direct comparison with an authentic sample of cucurbitacin D(**1**) showed complete identity.

The second triterpene showed similar spectroscopic properties to those of cucurbitacin D. Its UV spectrum showed a shift with added base indicating the presence of a diosphenol group. Direct comparison with an authentic sample of cucurbitacin I(**2**) showed identity.

Comparison of the ^{13}C-NMR spectrum of cucurbitacin D(**1**) with I(**2**) allowed assignment of the carbonyl carbons. The signal at 212.6 ppm in cucurbitacin D



moved to 198.4 ppm in cucurbitacin I and hence must be assigned to the 3-carbonyl. The carbonyl signal at 203.4 ppm was assigned to C-22 because it is part of an α,β-unsaturated system. The most downfield carbonyl at 213.6 ppm in cucurbitacin D must then belong to the 11-carbonyl group. The signals in cucurbitacin D(**1**) for the 5- and 6-carbons (141.8 and 120.5 ppm respectively) were assigned by comparison with those for these positions in cholesterol [5]. The two remaining vinyl carbons signals at 120.5 and 155.3 ppm were assigned to C-23 and C-24 respectively. The latter assignments are more difficult to make for cucurbitacin I(**2**) because many of the signals fall close together (see Experimental).

The signal at 72.2 ppm in cucurbitacin D(**1**) was assigned to C-2 since it was not present in the spectra of cucurbitacin I(**2**). The assignment of the other carbinol carbons was more difficult since the signals occur so close together (see Experimental). Further interpretation of the ^{13}C-NMR data was not undertaken.

Both of these cucurbitacins proved to be inactive as seed germination inhibitors. The mother liquors from the fractions containing these triterpenes still showed the seed germination inhibitory activity. Further efforts to obtain crystalline material from these fractions has until now been unsuccessful. The seed germination inhibitory activity of the mother liquors was lost after standing several months at room temp. TLC comparison of the extracts with the known naturally occurring seed germination inhibitors, arbutin and coumarin indicated their abscence in the plant extracts.

The occurrence of cucurbitacins is largely associated with the family Cucurbitaceae although limited occurrence has been reported in two unrelated families: Cruciferae (*Iberis* sps.) and Scrophulariaceae (*Gratiola* sps.) [6]. None of these three families are closely related botanically to the Rosaceae. The occurrence of tetracyclic triterpenes has not heretofore been reported in the Rosaceae [7]. The unexpected occurrence of cucurbitacins in *P. tridentata* accounts for the very bitter taste of bitterbrush seeds.

EXPERIMENTAL

Seed of *P. tridentata* were collected at two sites; *ca* 8 km S. of Hermiston, Oregon along Highway 32 in blow sand and along the Metolius River near Camp Sherman in the Cascade Range. A further supply of seeds was obtained from Burt McConnell, U.S. Forest Service. NMR data were taken at 60 MHz in $CDCl_3$. The integrated areas were consistent with the assignments.

Bioassay Seed germination inhibitory properties of fractions were determined by applying extracts to filter paper in divided covered plastic trays. Lettuce seeds were placed on the dampened filter paper and allowed to germinate with appropriate controls. The percentage of seeds germinating was an index of the inhibitory activity in the extract.

Isolation. The ground seeds were defatted with hexane and extracted with $CHCl_3$, EtOAc, Me_2CO, MeOH and H_2O. The bulk of the inhibitory properties occurred in the EtOAc extracts. These extracts contained large amounts of pigment. These extracts were chromatographed on polyamide. Fractions eluted with Me_2CO contained the inhibitory properties. The Me_2CO fractions were rechromatographed on polyamide. The Me_2CO fractions were recombined according to their TLC composition The active fractions at this point were a nearly clear gum. Work-up of these fractions by crystallization gave cucurbitacin D(**1**); mp 157–158.5° from H_2O; ν_{max}^{KBr} cm^{-1}; 3800–3230, 1720, 1695; λ_{max}^{EtOH} nm: 228.5; NMR: δ7.08, 6.75 (AB *doublet*, J = 17 Hz, H-24 and H-23), 5.73 (unresolved H-6), 4.32 (unresolved H-2 and H-16), 3.80–0 (unresolved); ^{13}C-NMR, 213.5 (C-11), 212.6 (C-3), 203.4 (C-22), 155.3 (C-24), 141.6 (C-5), 120.5(C-6 and C-23), 72.2(C-2), 79.1, 71.1, 70.7(C-16, C-20, C-25) ppm. Further crystallization of the mother liquors gave cucurbitacin I(**2**), mp 142–149° (EtOAc–hexane); λ_{max}^{EtOH} nm: 229, 268; I(**2**), mp 142–149° (EtOAc–hexane); λ_{max}^{EtOH} nm: 229, 268; $\lambda_{max}^{EtOH-NaOH}$ nm 305; NMR: δ7.11, 6.53 (AB *doublet*, J = 17 Hz, H-24, and H-23), 5.83 (*d*, J = 2.7 Hz, H-1); 5.70(unresolved H-6), 4.36(unresolved H-16), 3.9–0.8(unresolved), ^{13}C-NMR: 213.3 (C-11), 202.9(C-22), 198.4(C-3), 155.2(C-24), 145.2(C-2), 137.2(C-5), 115.2(C-1), 78.5, 70.9, 70.5(C-16, C-20, C-25), 120.8, 119.8(C-23, C-6) ppm

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