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SESQUITERPENE LACTONES OF SAGEBRUSH: THE STRUCTURE OF ARTECANIN

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Key Word Index—*Artemisia cana*, Compositae; sagebrush; sesquiterpene lactones; guaianolides, desacetylmatricarin; artecanin; canin; germacranolide, artevasin.

Abstract—*Artemisia cana* Pursh ssp. *cana* collected in Montana contained desacetylmatricarin, canin, artecanin and artevasin. Artecanin has been shown to have the structure **3** on the basis of the physical and spectral properties. The structure of canin has been revised to **2**.

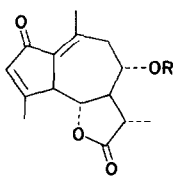
INTRODUCTION

The sesquiterpene lactones of *Artemisia cana* Pursh ssp. *cana* were studied as a part of this laboratory's program on the chemical investigation of sagebrush in Montana [1–3]. Chromatography of the chloroform extract of aerial parts of the plants collected in Montana gave desacetylmatricarin (**1**), canin (**2**), artecanin (**3**) and artevasin (**4**). The same plant species collected in Wyoming is known [4] to contain lactones **1**,**2**,**3** and ridentin (**5**). Though structures have been assigned to **1**,**2**,**4** and **5** [4–7] to our knowledge there has been no report on the structural elucidation of artecanin (**3**). Artecanin has now been shown to have structure **3** on the basis of spectral studies, which also permitted the revision of the structure of canin to **2**.

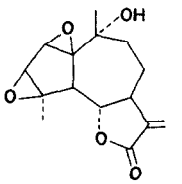
RESULTS AND DISCUSSION

The identity of artecanin isolated in the present investigation to the artecanin reported from Wyoming sample [4] was suggested by similar mps and Co-TLC in two different solvent systems, with an authentic canin-artecanin mixture.

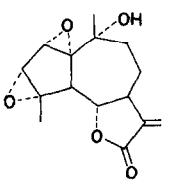
Artecanin, mp 244–245° $[\alpha]_D^{23}$ + 26·6° (EtOH,*C* 0·82) analyzed for $C_{15}H_{18}O_5$. Like canin the MS of artecanin showed a parent peak at *m/e* 278 and a base peak at *m/e* 111. It also gave a positive epoxide test [4,8] and showed IR peaks for OH (3436 cm^{-1}), γ-lactone (1745 cm^{-1}) and C-O-C (1156 cm^{-1}) groups.



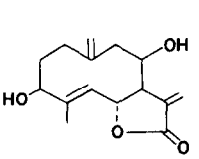
(**1**) R = H
(**1a**) R = Ac



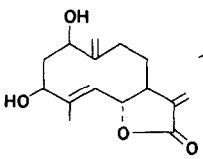
(**2**)



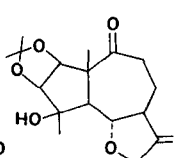
(**3**)



(**4**)



(**5**)



(**6**)

Artecanin gave a mono-trimethylsilyl derivative which showed no hydroxyl peak in its IR spectrum indicating the presence of one hydroxyl group. The tertiary nature of the hydroxyl group was evident by its resistance to acetylation with pyridine–acetic anhydride, a normal acetylating agent.

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Table 1. NMR spectral data of canin and artecanin*

Location of proton(s)	Canin	Artecanin
C-2 and C-3	3·43 (*d*, 1·5), 3·27 (*d*, 1·5)	3·53 (*d*, 1·0), 3·33 (*d*, 1·0)
C-4 —Me	1·45, *s*	1·46, *s*
C-5	2·35 (*d*, 11·5)	2·87 (*d*, 10·5)
C-6	4·42 (*dd*, 11·5, 9·5)	3·97 (*t*, 10·5)
C-7	*m*x	*m*x
C-10 —CH_3	0·98, *s*	0·97, *s*
C-11 =CH_2	6·03 (*d*, 3·5), 5·48 (*d*, 3·5)	6·05 (*d*, 3·5), 5·46 (*d*, 3·5)
Misc	4·87, *br*, OH	5·03, *bs*, OH

* These data were obtained at 60 MHz in DMSO-d_6 solns using TMS as an internal indicator. Chemical shifts are quoted in δ (ppm). Figures in parenthesis denote coupling constants in Hz.

The hydroxyl group and the γ-lactone accounted for three of the five oxygen atoms in artecanin. The remaining two oxygen atoms appeared to be involved in epoxide functions.

The NMR spectrum (DMSO-d_6) of artecanin showed a low field pair of doublets at 6·05 and 5·46 ppm characteristic of α-methylene-γ-lactone protons (C-11 = CH_2), a triplet at 3·97 ppm (J 10·5 Hz) and a doublet at 2·87 ppm (J 10·5 Hz) which can be assigned to the C-6 and C-5 protons respectively. Narrow doublets at 3·53 and 3·33 ppm (J 1·0 Hz) can be assigned to the C-2 and C-3 protons. The C-4 and C-10-methyl groups appeared as singlets at 1·46 and 0·97 ppm respectively. The hydroxyl proton appeared at 5·03 ppm as a broad signal. These NMR features which are quite similar to those of canin [4] and all of the above data strongly suggest that artecanin is an isomer of canin, differing in the stereochemistry of the functional groups.

The chemical shifts for various groups in canin and artecanin are similar (see Table 1) except for those assigned to the C-5 and C-6 protons.

In canin (**2**) the C-5 proton signal appeared as a doublet (*J* 11·5 Hz) at 2·35 ppm, whereas in artecanin the same proton signal appeared as a doublet (*J* 10·5 Hz) at 2·87 ppm. The C-6 proton signal in canin appeared as a doublet of doublets (*J* 11·5, 9·5 Hz) at 4·42 ppm, whereas in artecanin it appeared as a triplet (*J* 10·5 Hz) at 3·97 ppm. The large coupling constants exhibited by signals corresponding to the C-5 and C-6 protons in both compounds indicate that the C-6 proton bears a transdiaxial relationship with both the C-5 and C-7 protons. Assuming that the C-7 side chain is β-oriented as in all known guaianolides [4,5,9], both canin and artecanin should possess the same stereochemistry at C-5, C-6 and C-7 as shown in **2** and **3**. However, as noted above in artecanin the C-5 proton appeared 0·52 ppm downfield as compared to that of canin (see Table 1), whereas the C-6 proton showed normal chemical shifts as exhibited by the same proton in many guaianolides [5,9]. This downfield shift of the C-5 proton in artecanin can be explained if the epoxide functions attached to C-1, C-2 and C-3, C-4 are α-oriented [10]. The normal position of the C-6 proton also indicates that the C-10 hydroxyl group is α-oriented*. Conversely, in canin, the C-6 proton signal was deshielded by 0·45 ppm as compared to that of artecanin and other guaianolides whereas the C-5 proton appeared at a normal position [5,9] suggesting that both the epoxide groups in canin are β-oriented [10] as shown in **2**. The deshielding of the C-6 proton in canin, which almost equals the deshielding of the C-5 proton in artecanin, appears to be solely caused by the β-oriented epoxide functions. This means that the hydroxyl group at C-10 of canin must be α-oriented as in artecanin because a β-oriented C-10 hydroxyl group would eclipse the C-6 proton and further deshield it [11].

Canin, $C_{15}H_{18}O_5$, isolated in our investigation, was identified by its mp 245–246°, $[\alpha]_D^{23}$ –30·5 (EtOH, *C* 0·67) and MS peaks *m/e* 278 (M^+) and *m/e* 111 (base peak) [4,7]. Further confirmation of its identity with canin isolated from the Wyoming sample [4], was obtained by Co-TLC with an authentic sample, comparison of NMR spectra and by its conversion to the known [4] acetonide (**4**).

Artevasin was identified by its NMR, IR, mp and mmp with an authentic sample [6]. Desacetylmatricarin was identified by its NMR, mp and acetylation to matricarin (**1a**).

EXPERIMENTAL

All mps are uncorr. Si gel G. was used for TLC and plates were visualized by spraying with conc. H_2SO_4 and heating.

Plant material. The sample of *A. cana* Pursh ssp. *cana* was collected near Laurel, Montana (T.1N., R.23E., Section 21) on June 7, 1971. A sample of this material, ACC-1971, is kept in the University of Montana Herbarium.

* Models show that if the C-10 hydroxyl group was β-oriented, it would eclipse the C-6 proton and deshield it from the normal position [4,5,9]. On the other hand, the α-oriented hydroxyl group has no effect either on the C-6 or the C-5 protons. All the deshielding of the C-5 proton in artecanin is therefore solely by the α-oriented epoxide groups.

Isolation of desacetylmatricarin A sample of dried twigs and foliage (1165 g) was extracted with 8 l $CHCl_3$ and processed in the usual manner [12] The resulting dark syrup (80 g) was dissolved in C_6H_6 and chromatographed on Si gel using C_6H_6 and C_6H_6–Et_2O of increasing polarity and collecting 200 ml aliquots The first 12 aliquots of C_6H_6 and the following 12 aliquots of mixed solvents (9 1) gave a complex gummy mixture having camphor and menthol aroma The next 8 aliquots of mixed solvent (8 : 2) gave crystals which were filtered, combined (on TLC basis) and recrystallized from $CHCl_3$–Et_2O to give 175 mg desacetylmatricarin mp 128–129° (solidified and remelted at 152–153°), IR ν_{max}^{Nujol} cm^{-1} 3510 (hydroxyl), 1775 (γ-lactone), 1680 (cyclopentenone), 1630 and 1610 (unsaturation)

Isolation of artecanin The next 5 fractions (33–37) eluted from the above column with solvent mixture (7 3) gave a solid which on crystallization from Me_2CO gave 220 mg of fine needles of artecanin, mp 244–245° (lit [4] 244°), $[\alpha]_D^{23}$ + 26 6° (*C* 0 82, EtOH), IR ν_{max}^{Nujol} cm^{-1} 3436 (hydroxyl), 1745 (γ-lactone) and 1156 (C-O-C) (Found C, 64 41, H, 6 60 $C_{15}H_{18}O_5$ required C, 64 73, H, 6 52%) Treatment of 50 mg artecanin with Tri-Sil reagent gave a monotrimethylsilyl derivative which was used for IR and NMR spectra

Isolation of canin Continued elution of the above column with the same solvent mixture (7 .3) for the next 15 aliquots gave a mixture of artecanin and canin (~ 3 g) which could not be separated by fractional crystallization or rechromatography The next 5 fractions eluted with the same solvent, however, had canin as a major component Repeated recrystallizations of this mixture from Me_2CO resulted in the isolation of 320 mg of pure canin mp 245–246° (lit [4] 244–246°), $[\alpha]_D^{23}$ − 30 3° (*C* 0 67 EtOH), lit [4]–35°), IR ν_{max}^{Nujol} cm^{-1} 3496 (hydroxyl), 1760 (γ-lactone), 1652 (unsaturation) and 1152 (C-O-C) This had the same R_f as the authentic canin in 2 different solvent systems (Found C, 65 18, H, 6 76 $C_{15}H_{18}O_5$ required C, 64 73, H, 6 52%)

Isolation of artevasin Continued elution of the column with C_6H_6–Et_2O mixture (1 1) and Et_2O alone gave no pure or mixed crystals Elution with EtOAc gave a fine crystalline precipitate Recrystallization of the material from Me_2CO gave 150 mg artevasin mp 208–210° (alone or in admixture with an authentic sample [6]), $[\alpha]_D^{23}$ + 177° (*C* 0 34 MeOH, lit [6] + 244°, *C* 0 75 EtOH), IR ν_{max}^{Nujol} cm^{-1} 3300 (hydroxyl) and 1754 (γ-lactone), the NMR was identical to authentic artevasin A comparison of the NMR spectrum of artevasin with that of ridentin showed a close similarity between these two compounds However, an ethanolic solution of artevasin did not give a blue color on heating with a few drops of concentrated HCl as reported for ridentin, and also the physical constants were different [7] Consequently, we believe that artevasin and ridentin are configurational isomers

Matricarin Desacetylmatricarin (50 mg) was acetylated with C_5H_5N and Ac_2O to give 40 mg of fine needles of matricarin mp 189–192° (lit [13] 190–191°), IR ν_{max}^{Nujol} cm^{-1} 1775 (γ-lactone), 1735 and 1225 (acetate), 1670 (cyclopentenone) and 1630 and 1610 (unsaturation) Further confirmation was obtained by NMR

Reaction of canin with acetone and conc H_2SO_4 A soln of 48 mg canin in 5 ml Me_2CO was treated with 2 drops of conc H_2SO_4 The reaction mixture was kept at room temp for 1 5 hr Solvent was removed under red pres and solid obtained was crystallized from CH_2Cl_2–Et_2O-petrol to give 38 mg of fine needles of **(6)** mp 245–247° (lit [4] 246–248), IR ν_{max}^{Nujol} cm^{-1} 3400 (hydroxyl), 1755 (γ-lactone) and 1677 (cycloheptanone) A similar reaction with artecanin gave a mixture of two compounds which could not be separated in sufficient amount for further investigation

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