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SESQUITERPENOID LACTONES OF *ARTEMISIA*. CONSTITUENTS OF *ARTEMISIA CANA* SSP. *CANA*. THE STRUCTURE OF CANIN

K. H. LEE, R. F. SIMPSON and T. A. GEISSMAN

Department of Chemistry, University of California, Los Angeles, California 90024,* U.S.A.

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Abstract—In addition to the known, 5,4′-dihydroxy-3,6,7,3′-tetramethoxyflavone, five sesquiterpenoid lactones have been isolated from *Artemisia cana* Pursh. ssp. *cana*. These include the new compounds canin, artecanin, and ridentin, and the known matricarin and deacetylmatricarin. The structure of canin, a new guaianolide, has been shown to be IV on the basis of chemical transformations and spectral evidence.

RESULTS AND DISCUSSION

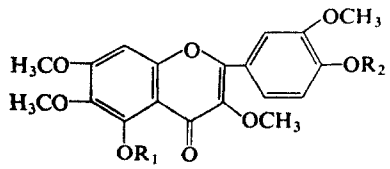
CONTINUING interest in chemical contributions to taxonomy and phylogeny in the *Artemisia*[1] has led us to examine the sesquiterpenoid constituents of *Artemisia cana* Pursh. subsp. *cana* (Compositae, tribe Anthemideae, section Seriphidium). Chromatography of the chloroform extract of the plant yielded fractions containing mixtures of crystalline substances. 5,4′-Dihydroxy-3,6,7,3′-tetramethoxyflavone (I), canin (IV), artecanin, deacetylmatricarin (Va) and ridentin were separated by careful rechromatography over silica gel. The structures of artecanin and ridentin, two new sesquiterpenoid lactones, are currently under investigation and will be reported upon in the future; that of canin is described in this paper.

The flavone (I), m.p. 177–179°, gives a red color with Mg and HCl and an olive-green color with $FeCl_3$. Its mass spectrum and elementary analysis, which corresponded to the formula $C_{19}H_{18}O_8$, along with the color reactions and i.r. and u.v. spectra, suggested that the compound was tetramethoxydihydroxyflavone. The NMR spectrum (measured in acetone-d_6) displayed four three-proton singlets at δ 3·37, 3·47, 3·50, and 3·52 (each 3H, s) (four OCH_3), δ 6·31 (1H, s, H-8), 6·55 (1H, d, H-5′, $J_{5',6'}=8{\cdot}5$ cps), 7·26 (1H, d, H-6′, $J_{5',6'}=8{\cdot}5$ cps) and 7·33 (1H, s, H-2′). Acetylation of I furnished the diacetate (II), m.p. 191–192°. The NMR spectrum of this compound is in agreement with structure II, revealing two three-proton singlets for the acetyl methyl groups at δ 2·32 and 2·48. The physical properties of the flavone and its acetate indicated that it was 5,4′-dihydroxy-3,6,7,3′-tetramethoxyflavone, which has been synthesized by Hörhammer *et al.*,[2] and was later isolated from *Matricaria chamomilla* L.[3] To further confirm the structure of the flavone, it was monomethylated with ethereal diazomethane, with the formation of III, identical with an authentic sample of artemetin obtained from *A. absinthium*.[4]

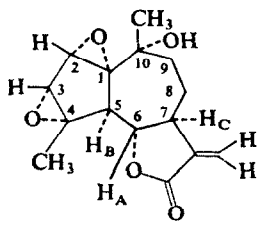
Canin (IV), m.p. 244–246°, $[\alpha]_D^{25}$ −35°, shows a molecular ion peak at *m/e* 278 and a base peak at *m/e* 112. Its elementary analysis agrees with a molecular formula of $C_{15}H_{18}O_5$.

* Contribution No. 2377 from the Department of Chemistry, UCLA.

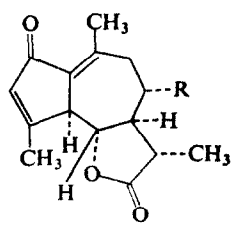
[1] T. A. GEISSMAN and M. A. IRWIN, *Progress in Organic Chemistry*, in press.
[2] L. HÖRHAMMER, H. WAGNER, E. GRAF and L. FARKAS, *Berichte* **98**, 548 (1965).
[3] R. HAENSEL, H. RIMPLER and K. WALTHER, *Naturwiss.* **53**, 19 (1966).
[4] T. A. GEISSMAN and T. E. WINTERS, *Tetrahedron Letters* 3145 (1968).



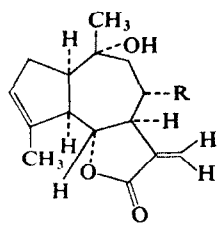
(I) $R_1 = R_2 = H$
(II) $R_1 = R_2 = COCH_3$
(III) $R_1 = H, R_2 = CH_3$



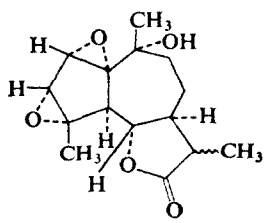
Canin (IV)



Deacetylmatricarin (Va) R = OH
Deacetoxymatricarin (Vb) R = H
Matricarin (Vc) R = $OCOCH_3$



Cumambrin B (VIa) R = OH
Cumambrin A (VIb) R = $OCOCH_3$
8-Deoxycumambrin (VIc) R = H



Dihydrocanin (VII)

Canin has an u.v. maximum at 208 nm (ϵ 9030), and i.r. bands at 1770 and 1660 cm^{-1}, characteristic of a γ-lactone conjugated with an exocyclic methylene grouping, a feature common to many sesquiterpenoid lactones of Compositae. The presence of a hydroxyl group was revealed by an i.r. band at 3475 cm^{-1}, disappearance of a signal at δ 4·81 (1H, s) in the NMR spectrum (in DMSO-d_6) of canin on addition of D_2O, and by an ion of *m/e* 260 (M-18) in the mass spectrum. That the hydroxyl group was probably tertiary was indicated by the fact that canin could not be acetylated with acetic anhydride in pyridine. The presence of the epoxy groups were indicated by a positive epoxide test[5] and by a strong and sharp band at 1155 cm^{-1} (—C—O—C—) in the i.r. spectrum.

The 100 mc NMR spectrum (in pyridine-d_5) of canin confirmed the presence of the α-methylene-γ-lactone grouping, which appeared as a pair of low-field doublets at δ 5·36 (1H, J=3 cps) and 6·18 (1H, J=3 cps). A typical ABC coupling pattern was observed for the protons of C-6 (H_A), C-5 (H_B) and C-7 (H_C). The proton H_A appears as a quartet

[5] J. M. ROSS, D. S. TARBELL, W. E. LOVETT and A. D. CROSS, *J. Am. Chem. Soc.* **78**, 4675 (1956).

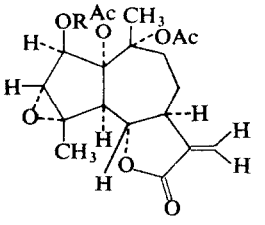
(J_{AB}=11 cps, J_{AC}=9·5 cps) centered at δ 4·45, the proton H_B as a doublet (J_{AB}=11 cps) at δ 2·27, and the proton H_C as a multiplet centered at δ 3·94. The coupling among these protons suggests a *trans*-axial arrangement; if the C_6-H configuration is β, as is likely, the hydrogen configurations are then H-5 α, H-6 β, and H-7 α. This configuration is reflected in the NMR signals for the corresponding protons of many sesquiterpenoid lactones of *Artemisia*, such as deacetylmatricarin (Va),[6,7] deacetoxymatricarin (Vb),[6,7] matricarin (Vc),[6,7] cumambrin B (VIa),[8,9] cumambrin A (VIb),[8,9] and 8-deoxycumambrin (VIc).[8] A pair of well-defined doublets at δ 3·28 (1H, J=1·5 cps) and 3·49 (1H, J=1·5 cps) is ascribed to the protons at C-2 and C-3, attached to the epoxide rings. The singlets at 1·22 (3H) and 1·62 (3H) are assigned to the C-10 methyl group and the C-4 methyl group, respectively.[10]

Catalytic hydrogenation of canin in ethyl acetate afforded dihydrocanin (VII), m.p. 220°, in almost quantitative yield. It had no u.v. absorption, and the NMR spectrum (in DMSO-d_6) showed the disappearance of the low-field doublets corresponding to the exocyclic methylene protons, and the appearance of the signal for a secondary methyl group at δ 1·08 (3H, d, J=6·5 cps). The other protons of dihydrocanin were seen as signals at δ 4·43 (1H, q, H-6, J_{AB}=11 cps, J_{AC}=9·5 cps); 2·12 (1H, d, H-5, J_{AB}=11 cps); 4·65 (1H, s, C-10 hydroxyl); 0·96 (3H, s, CH_3-10); 1·37 (3H, s, CH_3-4); and a pair of doublets at δ 3·25 (1H, J=1·5 cps) and 3·42 (1H, J=1·5 cps) corresponding to H-2 and H-3 of the two epoxide rings at C-1/C-2 and C-3/C-4.

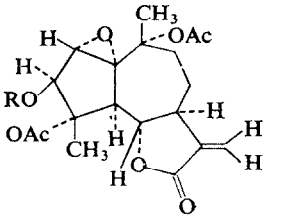
Acetylation of canin with isopropenyl acetate and *p*-toluenesulfonic acid provided a diacetate (VIIIa or VIIIb) whose composition was found to be $C_{26}H_{30}O_{10}S$, indicating that one of the two epoxide rings had been opened and a molecule of *p*-toluenesulfonic acid incorporated. Since there are no exchangeable protons in the NMR spectrum of the compound, the product must be a sulfonic acid ester. The crystalline product, which was very sensitive to heat, with m.p. 128–130° (dec.), gave an NMR spectrum which exhibited signals for an A_2B_2-type coupling pattern at δ 7·32 (2H, d, J=8·5 cps) and 7·82 (2H, d, J=8·5 cps), attributable to the aromatic protons of the tosyl group. A pair of AB-type doublets at δ 5·49 (1H, J=3 cps) and 6·18 (1H, J=3 cps) corresponded to the γ-lactone-α-methylene protons. Another pair of AB doublets at δ 3·89 (1H, J=2·5 cps) and 5·68 (1H, J=2·5 cps) were assigned to the protons H-2 and H-3. The signals appearing at δ 4·38 (1H, t, J=11 cps) and 2·69 (1H, d, J=11 cps) were attributed to H-6 and H-5. A sharp three-proton singlet, due to the tosyl methyl group, appeared at δ 2·44. The two acetyl groups at C-10 and C-4 appeared at δ 2·02 (3H, s) and 2·17 (3H, s) and the methyl groups at the same carbon atoms at 1·41 (3H, s) and 1·68 (3H, s), respectively. One of two possible structures, VIIIa and VIIIb, can be assigned to this compound; of these, the former is regarded as the most likely because the signals for the methyl groups at C-4 in VIII (a or b) and in II appear at nearly the same chemical shift value, indicating that the 3,4-epoxide is still present in VIII.

Treatment of canin with conc. H_2SO_4 in acetone resulted in the formation of an acetonide of the glycol formed by the *trans* ring opening of both epoxy groups to yield a *cis*-diol at H-2 and H-3, and the transformation of the C-10/C-1 glycol, formed by these ring openings, into a cycloheptanone, by the migration of the C-10 methyl group to C-1 and the generation

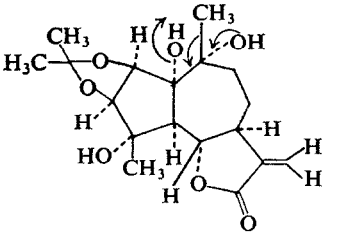
[6] T. A. GEISSMAN, T. STEWART and M. A. IRWIN, *Phytochem.* **6**, 901 (1967).
[7] E. H. WHITE and J. N. MARX, *J. Am. Chem. Soc.* **89**, 5511 (1967).
[8] M. A. IRWIN and T. A. GEISSMAN, *Phytochem.*, in press.
[9] J. ROMO, A. ROMO DE VIVAR and E. DÍAZ, *Tetrahedron* **24**, 1 (1968).
[10] These chemical shifts are almost identical with those of the C-10 (1·18 δ) and C-4 (1·60 δ) methyl groups of cumambrin B epoxide prepared in the course of structural elucidation of cumambrin B, as reported by Romo *et al.* (Ref. 9).



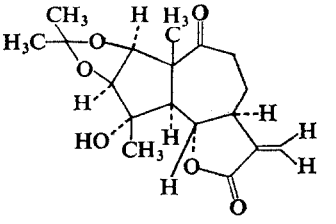
(VIIIa) R = *p*-Tos



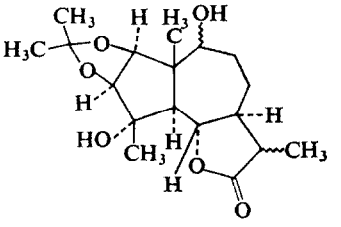
(VIIIb) R = *p*-Tos



(IX)



(X)



(XI) R_f 0·47

(XII) R_f 0·38

of a carbonyl group at C-10. The course of this rearrangement is that shown in IX → X. The product (X) thus formed had m.p. 246–248°, showed a molecular ion peak at *m/e* 336 in the mass spectrum, and analyzed for $C_{18}H_{26}O_6$. The NMR spectrum of X revealed the isopropylidene methyl, C-4 methyl, and C-1 methyl groups as sharp singlets at δ 1·44 (6H), 1·53 (3H), and 1·31 (3H), respectively. A pair of symmetrical AB-type doublets at δ 4·72 (1H, J=4·5 cps) and 4·34 (1H, J=4·5 cps) were assigned to H-2 and H-3. The γ-lactone-α-methylene protons displayed the characteristic well-defined doublets at δ 5·49 (1H, J=3 cps) and 6·22 (1H, J=3 cps), and the signals at δ 4·37 (1H, t, J=11 cps) and 2·62 (1H, d, J=11 cps) are assigned to H-6 and H-5. The presence of a seven-membered ring keto group in X was suggested by a prominent i.r. band at 1685 cm^{-1}, and the presence of the carbonyl group was confirmed by examination of the optical rotatory dispersion curve, which showed a positive Cotton effect (in CH_2Cl_2): $[\Phi]_{360}$ +1462°, $[\Phi]_{342}$ +2016°, $[\Phi]_{331}$ +3024°, $[\Phi]_{323}$ +3780°, $[\Phi]_{317}$ +3626°, $[\Phi]_{314}$ +3906° (peak), $[\Phi]_{303}$ +2016°, $[\Phi]_{292}$ −1008°, $[\Phi]_{278}$ −2419° (trough), $[\Phi]_{266}$ −1008° and $[\Phi]_{251}$ −3024°. Further confirmation of the presence of the carbonyl group was obtained by $NaBH_4$ reduction of X, which afforded a mixture of two dihydro compounds (XI and XII) (in the approximate ratio 2:1) with R_fs 0·47 and 0·38, respectively, on TLC. Separation of the mixture was achieved by column chromatography over silica gel. The faster-moving spot, R_f 0·47 (XI), showed the complete absence of ketonic carbonyl absorption in the i.r. Its NMR spectrum showed a doublet at δ 1·19 (3H, J=6·5 cps,

secondary methyl group of γ-lactone), and the following additional signals: 4·24 (1H, q, $J=11$ cps, $J=9·5$ cps, H-6), 2·52 (1H, d, $J=11$ cps, H-5), 1·30 (3H, s, CH_3-4), 3·62 (1H, broad s, C-10 hydroxyl),[11] 1·50 (3H, s) and 1·48 (3H, s) (isopropylidene methyl groups). The upfield shifts of the methyl group at C-1 (δ 1·09, 3H, s) and H-2 (δ 4·26, 1H, d, $J=4·5$ cps) in XI, compared with those in the NMR spectrum of X, in which the C—CH_3 signal appeared at δ 1·31 and H-2 at δ 4·72, further indicated that the C-1 methyl group in quaternary and, in X, adjacent to the C-10 carbonyl group. Support for this suggestion is found in the nature of the signals for H-2 and H-3 in X and XI. Both appear as pairs of AB-type doublets, but those in the latter are closer together as a result of a much more nearly similar magnetic environment compared with those of the former. The slower-moving spot of the mixture of reduction products, R_f 0·38 (XII), showed an i.r. spectrum almost superimposable upon that of XI, the only differences being seen in the hydroxyl absorption region. Compound XII is considered to be the epimer of XI.

A consideration of the evidence described in the foregoing leads to the assignment of the structure IV to canin. The co-occurrence of canin and matricarin is noteworthy, for the process of oxidative elaboration at positions, 10, 1, 2, 3 and 4 in matricarin could be regarded as the culmination of oxidative processes of which canin represents an intermediate stage.

The configuration at the γ-lactone ring junction of canin was deduced from a direct comparison of its circular dichroism curve with the results obtained from a recent systematic study of the circular dichroism of sesquiterpene lactones.[12] Canin and X both exhibit negative Cotton effects for the lactone ring (canin, in methanol: $[\theta]_{250}$ −2850°; X, in dichloromethane: $[\theta]_{250}$ −2290°) indicating that both possess C-6/C-7 *trans*-fused lactones with H-6 β and H-7 α. These results are consistent with those deduced from the coupling constants of H-5, H-6 and H-7 in the NMR spectra, as described above. Models show that the dihedral angle between H-6 and H-5 is approximately 180°, supporting the *trans*-axial relationship between these two protons. The formation of the acetonide (X) through the *trans* ring opening of the epoxides requires a *cis*-glycol at H-2 and H-3. Coupled with the facts that the naturally occurring guaianolides isolated from *Artemisia* so far all possess β-oriented C-10 methyl groups, these considerations lead to the representation of the complete stereochemistry of canin as in IV.

Deacetylmatricarin (Va) was isolated from chromatographic fractions after the removal of mixtures of canin and artecanin. It showed an u.v. maximum at 255 nm (ε 13,060) (characteristic of the dienone system) and NMR signals at δ 1·25 (3H, d, $J=6·5$ cps, secondary methyl group of γ-lactone), 2·31 (3H, s), 2·21 (3H, broad s) (two vinyl methyl protons at C-10 and C-4) and 6·11 (1H, broad s) (H-3). The correspondence of the properties of this compound with those described in the literature, and its conversion into matricarin by acetylation, established its identity. In an extraction of another collection of *A. cana* ssp. *cana*, obtained from the same locality, matricarin itself was also isolated, along with several of the compounds described above.

EXPERIMENTAL

M.ps were determined in capillary tubes, and are corrected. Optical rotations were determined in $CHCl_3$. U.v. spectra were determined in 95% ethanol on a Cary Model 14 spectrophotometer. I.r. spectra were determined in Nujol mulls on a Perkin-Elmer Model 237 spectrophotometer. NMR spectra were determined on a Varian Associates A-60 instrument in $CDCl_3$ using tetramethylsilane as the internal standard; s refers to singlet, d to doublet, t to triplet, q to quartet and m to multiplet. Mass spectra were determined on an

[11] This signal disappeared upon addition of D_2O.

[12] T. G. WADDELL, W. STÖCKLIN and T. A. GEISSMAN, *Tetrahedron Letters*, in press.

A.E.I. MS-9 instrument at 70 eV using direct insertion. Optical rotatory dispersion (ORD) and circular dichroism (CD) curves were determined on a Cary Model 60 and a model 6002 spectropolarimeter. Silica gel for column chromatography refers to Baker A.R. No. 3405 and silica gel for TLC refers to Merck Silica Gel G, developed with $CHCl_3$–acetone (3:2) and visualized by spraying with conc. H_2SO_4 and heating.

Extraction of Artemisia cana *subsp.* cana

The *A. cana* subsp. *cana* used was a collection made in the summer of 1968 in Albany County, Wyoming.[13] The air-dried plant material (4·1 kg) was ground in a Wiley mill and exhaustively extracted with $CHCl_3$ at ordinary temperature. Evaporation of the solvent *in vacuo* yielded a thick green-black tar. This was made slurried with methanol (3000 ml), and shaken with *n*-hexane (4000 ml) and water (1000 ml). The aqueous layer was washed well with hexane and the hexane layer was reextracted with water. The combined aqueous extracts were evaporated under reduced pressure to yield a dark greenish-brown residue (220 g). TLC showed that this crude extract was a mixture of many components.

Isolation of 5,4′-Dihydroxy-3,6,7,3′-tetramethoxyflavone, Canin, Artecanin, Deacetylmatricarin and Ridentin

The above crude residue was taken up in 1 l. of $CHCl_3$ and chromatographed on 2 kg of silica gel (13 × 47 cm). The column was successively eluted with benzene, benzene–$CHCl_3$, $CHCl_3$, $CHCl_3$–acetone, acetone, and acetone–methanol. 40 1 l. fractions were collected and the composition of the fractions determined by TLC. The first benzene eluate (fractions 1–7) contained only traces of low-melting (*ca.* 75°) waxes. The subsequent fractions (8–25) obtained from benzene–$CHCl_3$, $CHCl_3$ and $CHCl_3$–acetone (9:1) eluates contained mainly material giving a yellow spot on TLC, together with a mixture of other compounds. The $CHCl_3$–acetone (6:1) and $CHCl_3$–acetone (3:1) eluates (fractions 26–34) (108 g) afforded a mixture of three components. A mixture of compounds with lowest R_fs was finally obtained from $CHCl_3$–acetone (3:1) and acetone eluates (fractions 35–39) (50 g). The acetone–methanol eluate (fraction 40) contained only traces of polar substances and were not investigated further.

The above fractions were worked up to yield the following:

(*a*) *5,4′-Dihydroxy-3,6,7,3′-tetramethoxyflavone* (*I*). A dark brownish syrup (57 g) was obtained from fractions 8–25 after the removal of solvent. A small portion of this syrup was rubbed with anhydrous ether. The yellow, crystalline solid that separated was collected by filtration and recrystallized from acetone to yield yellow needles: m.p. 177–179°; λ_{max} 353 (ϵ 46,900), 271 (sh.) (ϵ 34,900) and 257 nm (ϵ 39,000); upon addition of NaOH it exhibited λ_{max} 403, 272 and 251 (sh.) nm; i.r. bands at 3350 (broad, OH), 1648 (C=O), 1605, 1585 (phenyl grouping), 1280, 1270, and 1220 cm^{-1}. The mass spectrum showed ions at *m/e* 374 (M^+) (base peak), 359 (M-15) and 331 (M-15-28). (Found: C, 61·09; H, 4·95. Calc. for $C_{19}H_{18}O_8$: C, 60·96; H, 4·85%.)

(*b*) *Canin* (*IV*). The dark-brown syrup (108 g) resulting from fractions 26–34 was dissolved in $CHCl_3$ and rechromatographed on silica gel (1·3 kg, 8·5 × 84 cm). Elution with $CHCl_3$ and a mixture of $CHCl_3$–EtOAc (3:1) provided mainly the flavone (**I**). Elution with $CHCl_3$–EtOAc (1:1) (3000 ml) gave three fractions (A, B, C; see below). The initial 1000-ml eluate (fraction A) yielded a yellowish-brown syrup which was treated with a small amount of anhydrous ether and allowed to stand overnight. The colorless prismatic needles deposited were collected (700 mg) and recrystallized from acetone to give canin which, after a second recrystallization from acetone, was obtained as fine colorless needles, m.p. 244–246°; $[\alpha]_D^{25}$ −35° (*c* = 1, pyridine). The relevant spectral (u.v., i.r., NMR, CD, MS) characteristics have been described in the text. (Found: C, 64·85; H, 6·72. $C_{15}H_{18}O_5$ required: C, 64·73; H, 6·52%.)

(*c*) *Artecanin.* A subsequent 1000-ml eluate (fraction B) afforded a brown syrup which was seeded with a crystal of canin and allowed to stand overnight. The resulting crystalline deposit was filtered and washed with anhydrous ether. Recrystallization from acetone yielded colorless crystals (1·3 g), m.p. *ca.* 210–215°. TLC indicated that this was a mixture of two compounds and contained mostly canin. The separation of this mixture was accomplished by rechromatography on silica gel with elution with a mixture of $CHCl_3$–EtOAc–acetone to give, besides canin, a small amount of artecanin as colorless needles, m.p. 244°. The structure of this compound is under investigation.

(*d*) *Deacetylmatricarin* (*Va*). The final 1-l. eluate (fraction C) provided a brown syrup which was seeded with canin to yield a colorless crystalline mixture (854 mg), m.p. *ca.* 210°. Examination of this material by TLC showed that it contained canin and artecanin. The mother liquor, after removal of the crystalline material, was allowed to stand overnight. The resulting crystalline deposit was collected and recrystallized from ethyl acetate to give colorless needles (2·05 g), m.p. 127–129°; λ_{max} 255 nm (ϵ 13,060); i.r. bands at 3500–3250 (broad and strong, OH), 1765 (γ-lactone), 1680 (cyclopentenone), 1635 and 1610 (C=C). It failed to depress the m.p. of an authentic sample of deacetylmatricarin, and the i.r. spectra were identical. Upon acetylation it gave matricarin, identified by comparison with authentic material.

(*e*) *Ridentin.* The dark-brown syrup obtained from the $CHCl_3$–acetone (3:1) eluate of the first column (fractions 35–37) yielded a mixture of oily substances. All attempts to purify these substances were unsuccess-

[13] The authors are grateful to Professor R. O. Asplund, University of Wyoming, for his kindness in procuring plant material for us. The identifications were authenticated by Dr. A. A. Beetle, and represent material described by him in Wyoming Range Management, Issue No. 172, 1963.

ful. The acetone eluate (fractions 38–39) afforded a colorless crystalline residue (50 mg). Recrystallization from acetone provided fine colorless crystals, m.p. 204–210°. A mixed m.p. determination and the TLC behavior of this compound showed it to be identical with ridentin, a new lactone which has been isolated from several other *A. tridentata* sub-species[14] and whose structure is now under investigation.

5,4′-Diacetoxy-3,6,7,3′-tetramethoxyflavone (II). Acetylation of I with Ac_2O/NaOAc gave the diacetate as colorless needles from $CHCl_3$–EtOH, m.p. 191–192°;[2] λ_{max} 322 (ε 36,400) 246 (sh) (ε 31,900) and 233 (sh) nm (ε, 36,800). The i.r. spectrum showed bands at 1755 (acetyl), 1365 (CH_3 of acetyl), 1640 (C=O), 1625, 1590 and 1513 cm^{-1} (phenyl grouping), and the absence of hydroxyl absorption. The NMR spectrum showed signals at δ 3·79 (3H, s), 3·84 (3H, s), 3·88 (3H, s), 3·96 (3H, s) (aromatic OCH_3), 2·32 (3H, s) (acetyl-CH_3), 2·48 (3H, s) (acetyl-CH_3), 6·72 (1H, s) (H-8), 7·12 (1H, d, $J_{5',6'}=8·5$ cps) (H-5′), 7·62 (1H, d, $J_{5',6'}=8·5$ cps) (H-6′) and 7·68 (1H, s) (H-2′). The mass spectrum showed *m/e* at 458 (M^+). (Found: C, 60·29; H, 4·95. Calc. for $C_{23}H_{22}O_{10}$: C, 60·26; H, 4·84%.)

5-Hydroxy-3,6,7,3′,4′-pentamethoxyflavone (III). The flavone (I) (3 mg) in methanol was treated with excess ethereal CH_2N_2 at 0° for 2 hr. The product was recrystallized from methanol–water to give yellow needles, m.p. 155°. The identity of this compound was established by direct comparison (m.p., i.r., MS) with an authentic sample of artemetin.[4]

Dihydrocantin (VII). A solution of canin (50 mg) in ethyl acetate (5 ml) was hydrogenated in the presence of Adams' catalyst (20 mg) at atmospheric pressure. The product was worked up in the usual manner, and the crystalline residue was recrystallized from ethyl acetate to form colorless needles, m.p. 220°; i.r. bands at 3490 (OH) and 1770 cm^{-1} (γ-lactone). The mass spectrum showed *m/e* at 280 (M^+), 260 (M-18), and 112 (base peak). (Found: C, 64·04; H, 7·17. $C_{15}H_{20}O_5$ required: C, 64·27; H, 7·19%.)

Acetylation of Canin with Isopropenyl Acetate and p-*Toluenesulfonic Acid*

A solution of canin (50 mg) and *p*-toluenesulfonic acid (100 mg) in isopropenyl acetate (5 ml) was allowed to stand at room temp. for 3 hr. The reaction mixture was diluted with ether, washed with aqu. $NaHCO_3$ and water, and the solvent removed under reduced pressure without warming. The product was recrystallized from $CHCl_3$–ether to yield colorless needles (VIIIa or VIIIb), m.p. 128–130° (dec.); i.r. bands at 1775 (γ-lactone), 1740, 1365 (acetate), 1600, 1490 (phenyl grouping) and 1650 cm^{-1} (C=C), and no absorption in the hydroxyl region. The NMR spectrum is described above. (Found: C, 58·42; H, 5·66. $C_{26}H_{30}O_{10}S$ required: C, 58·05; H, 6·02%.)

Treatment of Canin with Acetone-concentrated Sulfuric Acid

To a solution of canin (150 mg) in acetone (5 ml) was added 3 drops of conc. H_2SO_4. The mixture was allowed to stand for 1·5 hr at room temp. and after evaporation of the solvent *in vacuo*, diluted with water and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, dried, and evaporated under reduced pressure to give a crystalline residue which was passed through a small column of silica gel (1·5 × 13 cm) in $CHCl_3$. The eluate (150 ml) afforded 110 mg of pale yellow crystals which were collected, washed with anhydrous ether, and recrystallized from CH_2Cl_2–ether to yield fine colorless needles (90 mg) of (X), m.p. 246–248°; $[\alpha]_D^{25}$ +14° ($c=0·5$ $CHCl_3$); i.r. bands at 3400 (OH), 1765 (γ-lactone) and 1685 cm^{-1} (cycloheptanone). The mass spectrum showed *m/e* at 336 (M^+), 321 (M-15) (base peak). The NMR spectrum is described above. (Found: C, 64·08; H, 7·23. $C_{18}H_{26}O_6$ required: C, 64·27; H, 7·19%.)

Reduction of (X) with Sodium Borohydride

A solution of X (75 mg) in methanol (3 ml) was stirred with an excess of $NaBH_4$ at room temperature for 2 hr. The mixture was evaporated *in vacuo*, diluted with water, and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried, and evaporated to give a colorless oil which was chromatographed over silica gel (1·5 × 12 cm). Elution with $CHCl_3$–EtOAc (5:1) (30 ml) provided a colorless glassy substance which showed only one spot with R_f 0·47 (20 mg) (XI) on TLC; i.r. (in chloroform) bands at 3600, 3580, 3480 (broad and strong) (OH) and 1765 cm^{-1} (lactone C=O), and the absence of ketonic carbonyl absorption. Further elution of the column with $CHCl_3$–EtOAc (1:1) yielded a mixture of two compounds with R_f 0·47 and R_f 0·38, respectively. The ethyl acetate eluate gave 5 mg of a colorless glassy compound with R_f 0·38 (XII) which also showed i.r. bands (in chloroform) at 3650 (broad, weak), 3570 (strong), 3450 (broad, strong) (OH) and 1765 cm^{-1} (γ-lactone) and the absence of ketonic carbonyl absorption.

Attempted Acetylation of Canin with Pyridine–Acetic Anhydride

A solution of canin (50 mg) in Ac_2O (2 ml) and dry pyridine (4 ml) was allowed to stand at room temperature overnight. Only starting material (42 mg) was recovered.

Matricarin (Vc) was isolated in another extraction of *A. cana* ssp. *cana*, and was identified by comparison with an authentic specimen.

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[14] Unpublished observations by M. A. Irwin.