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THE ISOLATION AND STRUCTURE DETERMINATION OF ARTEMISIIFOLIN, A NEW GERMACRANOLIDE FROM *AMBROSIA ARTEMISIIFOLIA* L. (COMPOSITAE)

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Abstract—We describe the isolation and structure determination of artemisiifolin (III), a new germacranolide from *Ambrosia artemisiifolia*. The structure assigned to artemisiifolin was previously ascribed to salonitenolide, a germacranolide from *Centaurea saloitana* Vis. Preliminary studies suggest that the structure of salonitenolide should be revised to formula II. The final proof that artemisiifolin corresponds to III was provided by its direct formation from cnicin (VII), a germacranolide from *Centaurea* species. Isabelin (I) was found to occur as a minor constituent with artemisiifolin in a few populations of *A. artemisiifolia*.

INTRODUCTION

IN CONNECTION with our continuing biochemical systematic investigation of the genus *Ambrosia* (Compositae),[2] we report here the isolation and structure determination of a new germacranolide from several collections of *A. artemisiifolia* L. from near Austin and Arnett, Texas, and Wilberton and Norman, Oklahoma. The collections from Austin also contained isabelin (I), a germacranolide previously encountered in *A. psilostachya* DC.[3,4]

NMR spectral analysis of syrups obtained from two small collections of two other species were in accord with the presence of artemisiifolin: (1) *A. acanthacarpa* Hook from Bakersfield, California, and (2) *A. psilostachya* from La Marque, Texas. A number of pseudoguaianolide-type sesquiterpene lactones have been isolated from other populations of *A. artemisiifolia*: coronopilin,[5] psilostachyin,[6] cumanin,[7] peruvin,[7] and dihydrocumanin.[7] The germacranolide dihydroparthenolide has also been reported from one population of *A. artemisiifolia*.[6]

The structure we assign to artemisiifolin (III) was previously ascribed to salonitenolide, a germacranolide from *Centaurea salonitana* Vis.[8] Although the matter is still under investigation, preliminary evidence which will be presented elsewhere suggests that salonitenolide corresponds to structure II.

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(*b*) T. J. MABRY, in *Phytochemical Phylogeny* (Edited by J. B. HARBORNE), Academic Press, London (1969).
[3] H. YOSHIOKA, T. J. MABRY and H. E. MILLER, *Chem. Commun.* 1679 (1968).
[4] H. YOSHIOKA and T. J. MABRY, *Tetrahedron* (in press).
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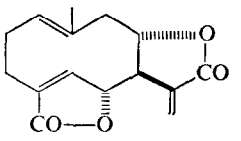
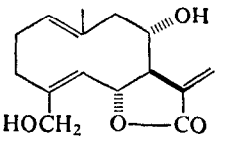
[7] T. H. PORTER and T. J. MABRY, *Phytochem.* **8**, 793 (1969).
[8] M. SUCHÝ, Z. SAMEK, V. HEROUT and F. SORM, *Coll. Czech. Chem. Commun.* **32**, 2016 (1967).



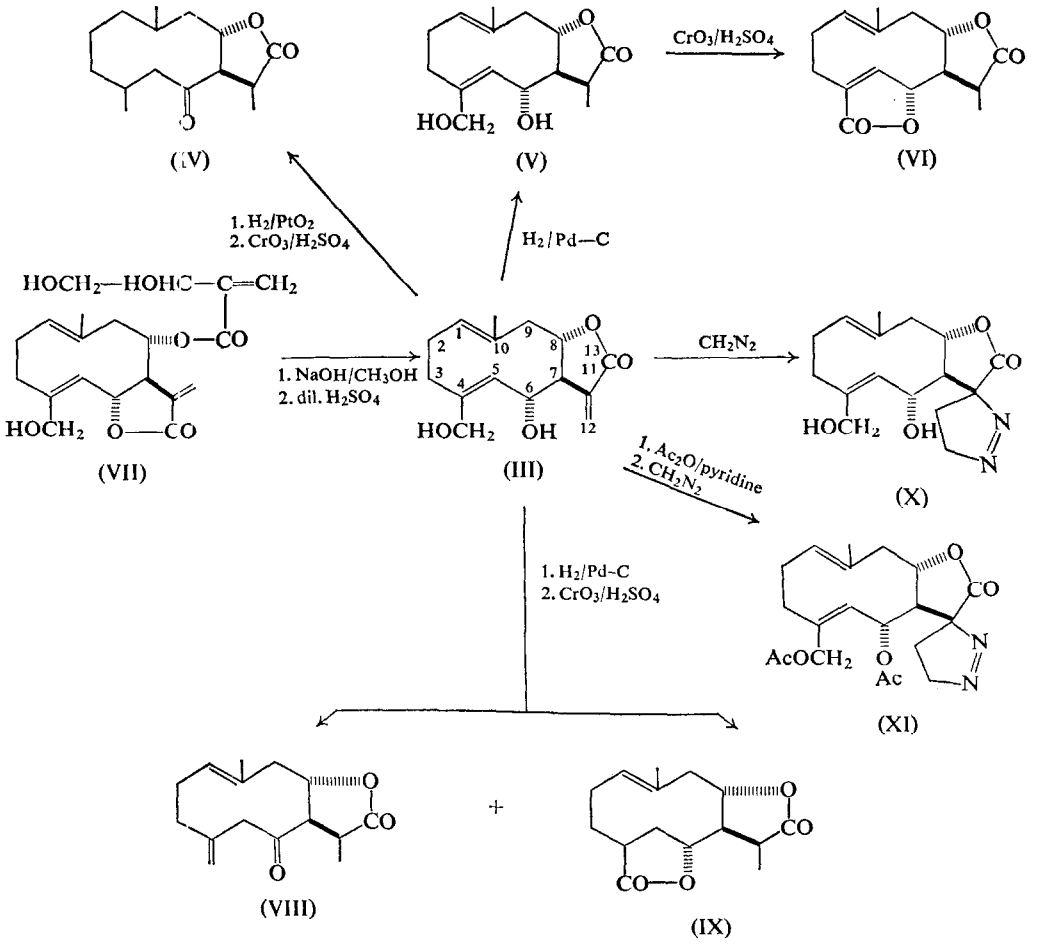
(I)



(II)

Isolation and Structure Determination of Artemisiifolin (III)

Chloroform extraction of dried leaves and stems of *A. artemisiifolia* collected near Austin, Texas, furnished in about 1·5 per cent yield a new substance, which we named artemisiifolin, and much smaller amounts of isabelin (II). The new compound, artemisiifolin, $C_{15}H_{20}O_4$, m.p. 131°, contained an α,β'-unsaturated γ-lactone [λ_{max} (EtOH): 208 nm, ϵ 14,000; i.r. bands at 1745 and 1640 cm^{-1}] and two hydroxyl groups (i.r. bands at 3380 and 3220 cm^{-1}; formation of a diacetate). The NMR spectrum of artemisiifolin in various solvents ($CDCl_3$,



acetone, DMSO) did not exhibit sharply resolved signals for most of the protons.[9] The most readily assignable signals were a singlet at 1·60[10] for one vinylic methyl group and complex one-proton signals at 6·25 and 6·10 typical for the two C-11 methylene group protons of the type found in the germacranolide lactone series.[3,4]

The germacranolide nature of artemisiifolin was initially established by exhaustive hydrogenation to hexahydrodeoxyartemisiifolin followed by oxidation to a substance identical with an authentic sample[11] of the 6-ketogermacrane derivative IV.[12] Milder hydrogenation conditions yielded dihydroartemisiifolin, m.p. 184°, $[\alpha]_D^{25}$ +94°, which was oxidized[13] to a dilactone identical with dihydroisabelin (VI). If one assumes no double-bond migration during the acidic steps, this result combined with the spectral findings established structure III for artemisiifolin.

The structure assigned to dihydroartemisiifolin (V) was previously ascribed to salonitolide[12] (literature value: m.p. 184°, $[\alpha]_D^{20°}$ +100°); although the two substances, dihydroartemisiifolin (V) and salonitolide, are apparently identical (i.r. spectroscopy),[11] an authentic sample of salonitolide was not available for direct comparison. It is noteworthy that salonitolide was not obtained by hydrogenation of salonitenolide,[8] a result which is in accord with our view that the structure of salonitenolide requires revision.

The final proof that structure III represents artemisiifolin was provided by the formation of material identical in all respects with the natural product by the alkaline hydrolysis of cnicin (VII).[14] Treatment of cnicin first with methanolic potassium hydroxide and then with dilute sulfuric acid afforded a substance identical with artemisiifolin (III). Although the alkaline treatment opens the lactone function in cnicin, it is known that the acidification step causes relactonization to C-8 rather than C-6.[15] Therefore the formation of artemisiifolin (III) from cnicin (VII) combined with the conversion of dihydroartemisiifolin to dihydroisabelin clearly establishes that artemisiifolin not salonitenolide corresponds to structure III.

Derivatives and Transformation Products of Artemisiifolin

In the course of the structural studies on artemisiifolin, a number of other interesting derivatives and transformation products were prepared and characterized: The material obtained by exhaustive hydrogenation of artemisiifolin with Pd-C as catalyst was oxidized directly with Jones reagent; two crystalline substances were obtained: compounds (VIII), $C_{15}H_{20}O_3$, m.p. 112–113° and (IX), $C_{15}H_{20}O_4$, m.p. 151°. The structure of VIII was clear

[9] We have observed that germacranolides lactonized to C-8 often give poorly resolved NMR spectra; these compounds, artemisiifolin, chamissonin (see M. F. L'HOMME, T. A. GEISSMAN, H. YOSHIOKA, T. H. PORTER, W. RENOLD and T. J. MABRY, *Tetrahedron Letters*, 3161, 1969), and isabelin[3,4] unlike the germacranolides lactonized to C-6, appear to exist in solution at room temperature in more than one conformational form. However, acetates of the C-8 lactonized germacranolides give good NMR spectra suggesting that they are held in one conformation.

[10] All chemical shift values are reported in p.p.m., δ-scale.

[11] We are grateful to Dr. V. Herout for an authentic sample of the 6-ketogermacrane derivatives (IV) and for the i.r. spectrum of salonitenolide.

[12] M. SUCHÝ, V. HEROUT and F. SORM, *Coll. Czech. Chem. Commun.* **30**, 2863 (1965).

[13] C. DJERASSI, R. R. ENGLE and A. BOWERS, *J. Org. Chem.* **21**, 1547 (1956).

[14] M. SUCHÝ, V. HEROUT and F. SORM, *Coll. Czech. Chem. Commun.* **27**, 2398 (1962). The absolute configurations at C-6, C-7, and C-8 in cnicin and related compounds were recently reversed: M. SUCHÝ, Z. SAMEK, V. HEROUT and F. SORM, *Coll. Czech. Chem. Commun.* **33**, 2238 (1968). Moreover, recent work by Z. SAMEK, M. HOLUB, V. HEROUT and F. SORM (*Tetrahedron Letters*, 2931 (1969)) indicate that the acyl moiety of cnicin has the structural features shown in formula VII and is attached to C-8 rather than C-6.

[15] B. DROZDZ, M. HOLUB, Z. SAMEK, V. HEROUT and F. SORM, *Coll. Czech. Chem. Commun.* **33**, 1730 (1968). We have independently observed with a number of germacranolides that relactonization to C-8 rather than C-6 is preferred.

from NMR spin-decoupling experiments and from the formation of IV upon hydrogenation. Compound IX was shown to be identical with tetrahydroisabelin.[3,4]

Treatment of artemisiifolin and diacetylartemisiifolin with diazomethane yielded the corresponding pyrazoline derivatives (X and XI, respectively), $C_{16}H_{22}N_2O_4$, m.p. 147° and $C_{20}H_{26}N_2O_6$, m.p. 156°.

TABLE 1. NMR DATA FOR ARTEMISIIFOLIN AND DERIVATIVES[a]

Compound	H-1	H-8	H-6	C_{11}-CH_3	C_{10}-CH_3	Misc.
III					1·60s	6·25c[b]; 6·10c[b]
IV		4·76c		1·28d (6)		2·80c[c]
V[d]				1·21d (7)	1·37	
VI	4·88c	4·40c	5·04c	1·44d (7)	1·61d (2)	6·92c[e]
VIII	5·41c	4·61c		1·30d (6)	1·50d (1)	5·04c[g]
IX	5·30c				1·68s	
X					1·41s	
XI	5·58c				1·58s	1·84s[f]; 2·08s[f]

[a] All spectra were recorded on a Varian A-60 Spectrometer in $CDCl_3$ unless otherwise noted, and δ values are given in p.p.m. relative to internal tetramethylsilane as reference. Signals are described as follows: s, singlet; d, doublet; c, complex. Number in parentheses denote coupling constants in c/s.

[b] Exocyclic methylene protons.

[c] H-7.

[d] The spectrum of this material was recorded in $^2H_6$-DMSO.

[e] H-5.

[f] Acetyl.

[g] Exocyclic methylene protons at C_4.

EXPERIMENTAL[16]

Isolation of Artemisiifolin (III)

Collections of *Ambrosia artemisiifolia* L. (Compositae) from Austin (25 October 1965, Voucher No. 242608)[17] and Arett, Texas (17 October 1965, Voucher No. 241875), and Wilberton (25 August 1965, Voucher No. 265919) and Norman, Oklahoma, June 1966, Voucher No. 47280) yielded artemisiifolin. NMR spectra of syrups obtained from two small collections of other species indicated the presence of artemisiifolin: (1) *A. acanthacarpa* Hook from Bakersfield, California (21 October 1965, Voucher No. 241895 and (2) *A. psilostachya* DC. from La Marque, Texas (15 September 1965, Voucher No. 243655).

We describe here the isolation of artemisiifolin from the Austin, Texas, collections of *A. artemisiifolia*; these collections also contained isabelin (II)[3,4] as a minor constituent: The air-dried ground plant material (700 g) was extracted with $CHCl_3$ and worked up as previously described[18] except that EtOH was used instead of MeOH to dissolve the viscous greenish black residue obtained from the $CHCl_3$ extraction. Artemisiifolin (10·5 g) crystallized from the crude extract over a period of several days. Recrystallization of the crude material first from EtOAc and then from acetone–hexane gave analytically pure artemisiifolin, m.p. 131°; $[\alpha]_D^{25°}$ +54·6 (MeOH); λ_{max} (EtOH): 208 nm (ε 14,000); i.r. bands (nujol): 3380 and 3220 (hydroxyls), 1745 (γ-lactone) and 1640 cm^{-1} (double bonds). (Found: C, 68·10; H, 7·66; O, 24·35; mol. wt. (mass spectrum), 264. $C_{15}H_{20}O_4$ required: C, 68·18; H, 7·58; O, 24·42; mol. wt. 264.)

The mother liquor from the artemisiifolin yielded, after column chromatography (silica gel, ether solvent), 80 mg of crystalline material, m.p. 165°, identical in all respects with an authentic sample of isabelin (II).[3,4]

6-Ketogermacran-8,13-olide (IV)

A solution of 300 mg artemisiifolin in 10 ml of MeOH–HOAc (1:1) was hydrogenated with PtO_2 as catalyst at room temperature for 16 hr. After filtering off the catalyst, the solvent was evaporated to give a colorless

[16] Melting points are uncorrected. Analyses were determined by Dr. ALFRED BERNHARDT, Mikroanalytisches Laboratorium, Elbach über Engelskirchen, West Germany.

[17] All voucher specimens are deposited in The University of Texas Herbarium, Austin.

[18] T. J. MABRY, H. E. MILLER, H. B. KAGAN and W. RENOLD, *Tetrahedron* **22**, 1139 (1965).

syrup which was dissolved in 3 ml of acetone. This solution was treated with an excess of Jones reagent (CrO_3)[13] in an ice bath. The mixture was added to 250 ml of cold H_2O, and the resulting solution was extracted repeatedly with $CHCl_3$ and then EtOAc. The combined extracts were evaporated to a colorless syrup, which crystallized immediately. Recrystallization of the crude material from acetone–hexane gave 70 mg of colorless needles, m.p. 154°; $[\alpha]_D^{25°}$ −30·5° (MeOH); λ_{max} (EtOH): 205 nm (ϵ 260); i.r. bands ($CHCl_3$): 1770 (γ-lactone) and 1700 cm^{-1} (keto group). This material was identical in all respects with an authentic sample of 6-keto-germacran-8,13-olide (IV).[11] (Found: C, 71·27; H, 9·40; O, 19·21. $C_{15}H_{24}O_3$ required: C, 71·39; H, 9·59; O, 19·02.)

Dihydroartemisiifolin (V)

A solution of 500 mg artemisiifolin in 8 ml of MeOH was hydrogenated with 5% Pd-C as catalyst at room temperature until 50 ml of H_2 was taken up (after about 15 min). After filtering off the catalyst, the solvent was evaporated to a syrup, which was subsequently separated by preparative TLC (silica gel G, ether); the band at R_f about 0·25 was eluted with acetone. Evaporation of the solvent left a crystalline residue, which was recrystallized from acetone–ether–hexane: 55 mg; m.p. 183–185°; $[\alpha]_D^{25°}$ +94° (MeOH); λ_{max} (EtOH): 210 nm (ϵ 5200); i.r. bands (nujol): 3400 and 3250 (hydroxyls), 1740 (γ-lactone), and 1650 cm^{-1} (double bond). (Found: C, 67·58; H, 8·18; O, 23·98. $C_{15}H_{22}O_4$ required: C, 67·64; H, 8·33; O, 24·03.)

Dihydroisabelin (VI) from Dihydroartemisiifolin (V)

A solution of 600 mg dihydroartemisiifolin in 30 ml of acetone was oxidized with an excess of Jones reagent in an ice bath. After the addition of 400 ml of cold H_2O, the solution was extracted repeatedly with $CHCl_3$ and then EtOAc. The combined extracts were evaporated to a colorless syrup, which was separated by TLC (silica gel G, ether). Iodine vapor indicated four distinct bands: the second band from the top (R_f about 0·45) yielded 35 mg of crystals, m.p. 182–183° after recrystallization from acetone–hexane–$CHCl_3$; $[\alpha]_{365}^{25}$ −191° ($CHCl_3$); λ_{max} (EtOH): 209 nm (ϵ 15,300): i.r. bands (nujol); 1745 (γ-lactones) and 1640 cm^{-1} (double bonds). This material was identical in all respects with an authentic sample of dihydroisabelin (VI).

Germacrane Derivatives VIII and IX

A MeOH solution containing 1·8 g of artemisiifolin was hydrogenated in the presence of 5% Pd-C at room temperature until approximately 1·5 equivalents of H_2 were taken up. After filtering off the catalyst, the solvent was evaporated to a syrup, which was separated by TLC (silica gel G, ether). Iodine vapor showed three distinct regions: region A, R_f 0·9 (two spots); region B, R_f 0·7 (three or four overlapping spots); and region C, R_f 0·5 (one spot).

The material eluted from region A with acetone was oxidized with an excess of Jones reagent at ice bath temperature. Work-up of the oxidation mixture afforded a colorless syrup, which was separated by TLC (silica gel G, ether): yield 130 mg of VIII; m.p. 112–113° (after recrystallization from $CHCl_3$–hexane); $[\alpha]_D^{25°}$ +39·5° (MeOH); λ_{max} (EtOH): 207 nm (ϵ 4900); i.r. bands (nujol): 1765 (γ-lactone), 1695 (keto group), 1635 and 895 cm^{-1} (double bonds). (Found: C, 72·45; H, 8·12; O, 19·53. $C_{15}H_{20}O_3$ required: C, 72·55; H, 8·12; O, 19·33.)

Reduction of VIII in acetic acid–MeOH (1:4) with Pd-C as catalyst afforded material identical with the 8-ketogermacrane derivative (IV). The material from region B, after oxidation and work-up in the same manner as described above for the material from region A, afforded 25 mg of IX, m.p. 150–152°; $[\alpha]_{365}^{25}$ −67·6 ($CHCl_3$); λ_{max} (EtOH): 207 nm (ϵ 3700); i.r. band (nujol): 1750 cm^{-1} (γ-lactones). This material was identical in all respects with an authentic sample of tetrahydroisabelin (IX).

Artemisiifolin Pyrazoline (X)

A solution of 110 mg of artemisiifolin in 5 ml of $CHCl_3$ was treated overnight with an excess of CH_2N_2 in ether. Work-up of the reaction solution afforded (after recrystallization from acetone–hexane) 80 mg of X as needles, m.p. 146–147°; $[\alpha]_D^{25°}$ −201 (MeOH); λ_{max} (EtOH): 208 nm (ϵ 7600); i.r. bands (nujol): 3220 (hydroxyls), 1770 (γ-lactone) and 1640 cm^{-1} (double bonds). (Found: C, 62·64; H, 7·31; N, 9·05; O, 21·99. $C_{16}H_{22}N_2O_4$ required: C, 62·72; H, 7·24; N, 9·14; O, 20·89.)

Diacetylartemisiifolin Pyrazoline (XI)

A solution of 127 mg of artemisiifolin in 6 ml of Ac_2O and 19 drops of pyridine was allowed to stand at room temperature for 16 hr. The reaction mixture yielded a diacetyl derivative (by NMR) as a colorless syrup; the material was not obtained crystalline even after TLC separation. Treatment of the syrup with an excess of CH_2N_2 in ether yielded 35 mg of material: after recrystallization from CH_2Cl_2–hexane, m.p. 155–157°; $[\alpha]_D^{25°}$ −98° (MeOH); λ_{max} (EtOH); 207 nm (ϵ 8700); i.r. bands (nujol); 1760 (γ-lactone) and 1720 cm^{-1} (ester). (Found: C, 61·41; H, 6·79; N, 7·09; O, 24·69. $C_{20}H_{26}N_2O_6$ required: C, 61·52; H, 6·71; N, 7·18; O, 24·59.)

Artemisiifolin (III) from Cnicin (VII)

A solution of 1 g of cnicin in 20 ml of MeOH was warmed for 1 hr with methanolic KOH (2·5 g in 20 ml). Work-up of the reaction mixture was previously described[4] afforded (after recrystallization from EtOAc) 105 mg of crystals, m.p. 128–130°, which were identical by m.m.p., i.r. and NMR with artemisiifolin (III).

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