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SESQUITERPENE LACTONES. SOME NEW CONSTITUENTS OF *AMBROSIA* SPECIES: *A. PSILOSTACHYA* AND *A. ACANTHICARPA*

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Abstract—Three distinct populations of *Ambrosia psilostachya* and four specimens of *A. acanthicarpa*, two of them being seedling and mature plants from a single location, have been examined and their sesquiterpene lactones isolated. Different populations vary markedly in lactone content, and the seedling and mature forms showed a wide qualitative difference. A new lactone, cumanin-3-acetate, and cumanin diacetate, not before found in nature, were isolated from *A. psilostachya*. Seven other lactones, all known in *Ambrosia* species, were also isolated.

INTRODUCTION

STUDIES in this laboratory and elsewhere[2] have shown that variation of chemical constitution within a plant species is commonly encountered. Perhaps the most striking examples of the diversity between morphological characterization and chemical constitution are found in the genus *Ambrosia* (fam. Compositae, tribe Ambrosiaeae), in which the species *A. psilostachya* has been found to contain over fifteen sesquiterpene lactones distributed in groups of 1 (rarely) –5 or more in single populations.

Besides variations from one population to another (genetic), we have observed wide diversities in chemical composition of plants growing in the same location but differing in chromosome number,[3] and in plants of the same "population" collected in seedling and mature stages of growth. Indeed, it has been our experience that seldom do two separate populations of the same species show identity in chemical constitution. The present account is a description of studies of three specimens of *A. psilostachya* collected from three localities in Southern California, and of several *A. acanthicarpa* specimens, two of them representing seedling and mature stages of growth.[4]

RESULTS AND DISCUSSION

Ambrosia acanthicarpa (Hook.) Cov. is an annual roadside weed found widely distributed in coastal Southern California. An earlier study of this plant, collected in June in San Diego County, disclosed that the mature form is rich in chamissonin,[5,6] a sesquiterpene lactone first found in the closely related species *A. chamissonis*.[7] A later collection, made in March of

[1] Contribution No. 2262 from the Department of Chemistry, University of California, Los Angeles.
[2] H. E. MILLER, T. J. MABRY, B. L. TURNER and W. W. PAYNE, *Am. J. Botany* **55**, 316 (1968).
[3] T. A. GEISSMAN and S. MATSUEDA, *Phyotchem.* **7**, 1613 (1968).
[4] We are indebted to Dr. W. W. PAYNE for his identification of the specimens used in this study.
[5] T. A. GEISSMAN, R. J. TURLEY and S. MURAYAMA, *J. Org. Chem.* **31**, 2269 (1966).
[6] T. A. GEISSMAN and J. LEVY, *Phytochem.* **6**, 899 (1967).
[7] W. W. PAYNE, *J. Arnold Arbor.* **45**, 401 (1964); and by private communication.

the following year[8] yielded seedling plants which, surprisingly, contained no chamissonin but yielded three lactones, all previously isolated from other *Ambrosia* species. These were psilostachyin C (II),[9] confertiflorin (I), and deacetylconfertiflorin (III).[10] A reexamination of a second collection of the mature plant (June) showed that, as previously reported, it contains chamissonin as its principle lactonic constituent, but also contains psilostachyin C as well as at least one other sesquiterpene lactone, which was tentatively identified as confertiflorin, but which could not be isolated as a crystalline compound. No deacetylconfertiflorin was present.

Thin-layer chromatograms of total extracts of these two specimens confirmed in a qualitative way the above findings. Both plants contained from four to six easily discernable components, only one of which (psilostachyin C) was clearly present on both chromatograms. Chamissonin was not detectable on chromatograms of the seedling plant.

A third specimen of *A. acanthicarpa* (the mature plant), collected in metropolitan Los Angeles, was not greatly different from the mature specimen obtained in San Diego County. Comparison thin-layer chromatograms showed that both contained chamissonin as the predominant component, with smaller, and essentially identical, amounts of several other compounds.

Three specimens of *A. acanthicarpa*,[11] numbered BMA, BMA-a, and BMA-c (Voucher No. 6042) were examined by thin-layer chromatography. Of these, BMA and BMA-a were substantially identical with the Los Angeles specimen described above. The third (BMA-c) was strikingly different. It contained only traces of chamissonin, but a hitherto unobserved constituent appeared as an intense spot on the chromatogram. This compound was isolated by preparative TLC (79 mg from 32 g of plant material), and was found to be identical with a hitherto unreported compound, called by us artenovin, which, along with its acetate, had been isolated in this laboratory from two species of *Artemisia* (*A. nova* and *A. tripartita* ssp. *rupicola*).[12] Cumambrin B (artenovin) has the structure (X), and is of special interest in this connection because it is a guaianolide, for the bicyclic lactones of *Ambrosia* species are characteristically pseudoguaianolides, possessing the rearranged, non-isoprenoid structures with a methyl group at C-5 instead of C-4 (cf. I-IX).[13]

The occurrence in *Ambrosia* and in *Artemisia* of the same compound, and, more particularly, of a guaianolide, is of considerable phylogenetic interest, for Payne has pointed out the affinity between the ragweed group and the Heliantheae and Anthemideae. It should be added here that although studies of genera belonging to the tribe Heliantheae are still very few in number, the scanty information at hand[14] has disclosed no such chemical similarities between the Heliantheae and the Ambrosieae as the example of artenovin reveals between the Anthemideae and the Ambrosieae.

[8] That these seedlings were of the same "population" is certain, for they were found in the same location and were later seen in maturity.

[9] H. B. KAGAN, H. E. MILLER, W. RENOLD, M. V. LAKSHMIKANTHAM, L. R. TETHER, W. HERZ and T. J. MABRY, *J. Org. Chem.* **31**, 1629 (1966).

[10] N. H. FISCHER and T. J. MABRY, *Tetrahedron* **23**, 2529 (1967).

[11] Supplied by Dr. W. W. PAYNE, whose specimen designations are used for identification.

[12] Artenovin now appears to be identical with a compound called cumambrin B, isolated by J. ROMO, A. ROMO DE VIVAR and E. DIAZ from *Ambrosia cumanensis*. We are grateful to Dr. ROMO for a copy of the galley proof of their article (*Tetrahedron*, in press, 1968) describing the compound.

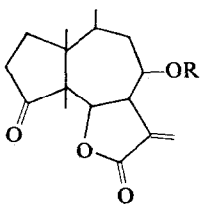
[13] Dr. J. Romo[12] has commented upon the probable biosynthetic implications of the co-occurrence of cumambrin A and the pseudoguaianolides in the same genus. In the case of *A. acanthicarpa*, cumambrin B and the germacranolide, chamissonin, occur in the same plant.

[14] For example, T. A. GEISSMAN and R. MUKHERJEE, *J. Org. Chem.* **33**, 656 (1968).

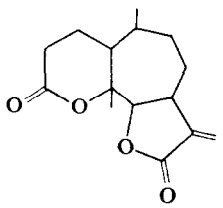
Ambrosia psilostachya DC. (western ragweed) is a common, widely distributed perennial, common to much of the western United States. We have examined specimens collected in several locations in Southern California, one of which, described earlier,[15] found in metropolitan Los Angeles, is constant in composition and contains chiefly coronopilin along with a trace of parthenin. Crude syrups prepared from extracts of this plant usually crystallize completely when seeded with coronopilin.

A specimen of *A. psilostachya*, collected in San Diego County at an elevation of about 2500 feet, was morphologically similar to the Los Angeles variety, but differs greatly in chemical composition. From this plant were isolated four sesquiterpene lactones, of which one was the 3-*O*-acetyl derivative of cumanin, hitherto unreported.

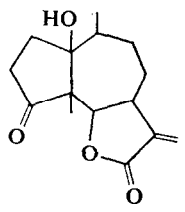
Cumanin-3-acetate (VII), m.p. 140–142°, $[\alpha]_D^{27}+141{\cdot}5°$ ($CHCl_3$, c=1·0), was readily identified by its spectral characteristics and by its conversion into the known cumanin diacetate.[16] In addition to cumanin-3-acetate, this specimen also contained cumanin (VI), coronopilin (IV), and parthenin (V), all of which are known constituents of several *Ambrosia* species.



(I) R = Ac
(III) R = H



(II)



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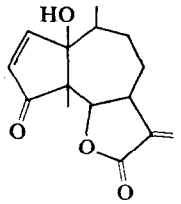
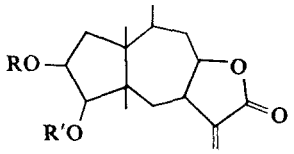
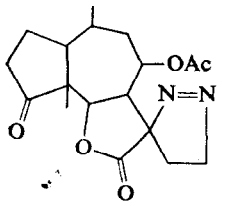


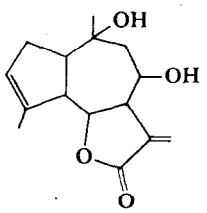
(V)



(VI) R = R′ = H
(VII) R′ = H; R = Ac
(VIII) R = R′ = Ac



(IX)



(X)

[15] T. A. Geissman and R. J. Turley, *J. Org. Chem.* **29**, 2553 (1964).
[16] J. Romo, P. Joseph-Nathan and G. Siade, *Tetrahedron* **22**, 1499 (1966).

The location of the acetyl group at the 3-position in the cumanin monoacetate was readily determined by examination of the NMR spectrum. Besides the familiar signals for the components of the lactone ring and for the secondary (at C-10) and tertiary (at C-5) methyl groups, the spectrum contained two one-proton signals for the hydrogen atoms of the *CH*-OAc and *CH*-OH groups. The lower-field signal, for the proton on the *CH*OAc group, was a multiplet, indicating coupling with the three protons at C-2 and C-4. The higher-field signal at δ 3·72 ppm ($J = 7{\cdot}5$ cps), for the proton on the *CH*OH group, was a doublet, as is to be expected for the location of the hydroxyl group at C-4, for this proton is coupled only to the proton at C-3. The magnitude of the coupling constant is in accord with the known[16] *cis*-relationship of the two hydroxyl groups in cumanin.

Cumanin was identified by its melting point and by the characteristics of its i.r. and NMR spectra. It was converted into the acetonide, the diacetate (VIII) and the diformate, the properties of all of which agreed with those reported.[16] Coronopilin and parthenin were identified by direct comparison with authentic specimens.

A third specimen of *A. psilostachya*, collected as a roadside weed at Santa Barbara, California, was morphologically indistinguishable from the Los Angeles and San Diego County specimens. It contained no coronopilin, but yielded cumanin, cumanin-3-acetate, and cumanin diacetate (VIII). Cumanin diacetate has not been reported as a natural compound. The above observations are summarized in Table 1.

TABLE 1. SESQUITERPENE LACTONES IN VARIOUS SPECIMENS OF *Ambrosia* SPECIES

Species	Locality	Age	Constituents (major underlined)
A. psilostachya	Los Angeles	Mature	*Coronopilin*, parthenin
	San Diego Co.	Mature	*Coronopilin*, *cumanin*, cumanin-3-acetate, parthenin
	Santa Barbara	Mature	*Cumanin*, cumanin-3-acetate, cumanin diacetate
A. acanthicarpa	San Diego Co.*	Seedling	*Confertiflorin*, deacetyl-confertiflorin, psilostachyin C
	San Diego Co.*	Mature	*Chamissonin*
	Los Angeles	Mature	*Chamissonin*, psilostachyin C
	—	Mature	Chamissonin, *artenovin*

* From identical locality.

EXPERIMENTAL

Voucher specimens are deposited in the U.C.L.A. Herbarium, except for those materials supplied by Dr. W. W. Payne.

Ambrosia acanthicarpa (*spring sample*)

3 kg of dried, ground *A. acanthicarpa* seedlings (No. 32167-ACAN-SD2) were extracted with $CHCl_3$ and the solvent evaporated. The tarry residue was dissolved in 200 ml of hot ethanol and 500 ml of hot water was added with vigorous stirring. The aqueous layer was separated, clarified by filtration through celite, and extracted with $CHCl_3$. Removal of the solvent left 26·5 g of a yellow-brown syrup, TLC analysis of which showed the presence of about 4 prominent components.

The crude material was chromatographed over silica gel (500 g, 5 × 51 cm), elution being carried out with benzene with increasing proportions of $CHCl_3$, and finally ether-methanol mixtures. Rechromatography of fractions grouped by TLC examination yielded, in order of appearance from the column, confertiflorin (I), psilostachyin C (II), and deacetylconfertiflorin (III).

Confertiflorin (I) had m.p. 146–147°; $[\alpha]_D^{24} + 51°$ (MeOH, c = 0·28) (reported[10] m.p. 145°, $[\alpha]_D^{26} + 25{\cdot}0°$ (MeOH, c = 5·0)). The NMR spectrum was in complete agreement with the structure assigned.[10] The mass spectrum (not previously described) shows the molecular ion at *m/e* 264 and a prominent peak at *m/e* 246

(M-18). Infrared absorption was observed at 3580 (OH), 1765 (lactone) and 1750 (cyclopentanone) cm^{-1}. (Calc. for $C_{17}H_{22}O_5$: C, 66·67; H, 7·19 . Found: C, 66·77; H, 7·30 per cent.)

Psilostachyin C (II) had m.p. 225° (reported,[9] m.p. 223–225°). It was identified by direct comparison with an authentic specimen, with which it was identical (m.p., mixed m.p., i.r., NMR spectra). (Calc. for $C_{15}H_{20}O_4$: C, 68·18; H, 7·58. Found: C, 68·33; H, 7·76 per cent.)

Deacetylconfertiflorin (III), had m.p. 145–6° (reported,[10] m.p. 145°). Its identity was established by acetylation to confertiflorin, the product of which was identical with the natural compound. The i.r. and NMR spectra were in complete accord with the structure assigned.

Monopyrazoline of Confertiflorin (IX)

An ether solution of the CH_2N_2 (from 400 mg of N-methyl-N-nitrosourea) was added to a solution of 114 mg of I in 20 ml of tetrahydrofuran. After 10 hr (at 5°) the solution was filtered and the solvent removed. The product, recrystallized from acetone-hexane had m.p. 158° dec. (Calc. for $C_{18}H_{24}O_5N_2$: C, 62·05; H, 6·93. Found: C, 62·29; H, 6·99 per cent.)

The NMR spectrum of the pyrazoline showed the singlet signal for the acetyl methyl group at δ 1·9 ppm, in contrast to the δ 2·1 ppm at which this methyl group is found in confertiflorin. This large deshielding effect of the pyrazoline ring is in agreement with position of the acetyloxy grouping at the 8-position, adjacent to the C-7/C-11 bond of the lactone ring.

A. acanthicarpa (*June sample*)

A 2·3 kg sample of mature *A. acanthicarpa* (No. 62467-ACAN-79) was extracted with $CHCl_3$ and the extract treated as described for the spring specimen. The final $CHCl_3$ extract yielded 67 g of crude syrup from which, upon trituration with a little ethyl acetate, 5·0 g of chamissonin crystallized. The remaining oily material was chromatographed on silica gel (600 g) with benzene, benzene-$CHCl_3$ and $CHCl_3$ as eluants. Although no crystallization occurred in any of the fractions, psilostachyin C and confertiflorin were identified by TLC analysis of early fractions. Later fractions, shown by TLC to contain chamissonin, were rechromatographed and the chamissonin-containing fractions combined.

Acetylation of all of the chamissonin, crystalline and noncrystalline, yielded 10·1 g of the readily crystallized and purified chamissonin diacetate, m.p. 177–178°, identified by comparison with an authentic specimen.[17]

Cumambrin B (X) from A. acanthicarpa (*BMA-c*)

A 32-g sample of *A. acanthicarpa* (W. W. Payne, No. 6042, sample BMA-c) was extracted with $CHCl_3$ in the usual way, and the extractives taken up in ethanol-water and then extracted with $CHCl_3$ to give a small quantity of yellow oil. This was subjected to preparative TLC (three 20 × 20 cm, 2-mm plate). 4 zones were detected by examining the plate under an u.v. lamp. These were removed from the plate and extracted with acetone. The lowest-R_f band yielded chamissonin; the second and fourth gave uncrystallizable oils; and the third gave 79 mg of a crystalline compound m.p. 176–177°. This was identified as artenovin (now cumambrin B) previously isolated in this laboratory from two species of *Artemisia*, by direct comparison (m.p., NMR, TLC) with an authentic specimen. A comparison (by J. Romo) of "artenovin" and its acetate with cumambrins B and A, respectively, showed them to be identical. Although Romo *et al.* report[12] the m.p. of cumambrin B as 87°, he has discovered (private communication) that this is a solvated form.

Ambrosia psilostachya (No. 62366-AMB), collected at an elevation of about 2500 ft on Highway 79 near Escondido, California, was extracted in the manner described above. From 4·2 kg of dried plant was obtained 76 g of final $CHCl_3$ extractable material as a yellow gum. Chromatography of this over 1100 g of silica gel, with solvent gradation from benzene through chloroform and acetone yielded a number of chromatographically distinct (TLC) fractions of which four could be obtained as crystalline materials.

Coronopilin (IV) (10·7 g) and *parthenin* (V) (0·31 g) were identified by comparison with authentic samples.

Cumanin-3-acetate (VII) (0·42 g) was crystallized from ethyl acetate and then from ether as colorless needles, m.p. 142–143°, $[\alpha]_D^{27}$ +147·5° (c=1·0, $CHCl_3$). (Calc. for $C_{17}H_{24}O_5$: C, 66·21; H, 7·85. Found: C, 66·39; H, 8·08 per cent.) The mass spectrum showed the molecular ion at *m/e* 308, with prominent peaks at 290 (M-18), 266 (M-42) and 248 (M-60).

Acetylation of cumanin-3-acetate yielded the known diacetate (VIII),[16] m.p. 105–107° (raised to 109° by sublimation).[18]

[17] Chamissonin is invariably difficult to crystallize, but acetylation of the intractable chamissonin-containing gums gives excellent yields of the diacetate.

[18] The melting points of cumanin and cumanin diacetate (but not the 3-acetate) are erratic and difficult to reproduce. Recrystallized from ethyl acetate-hexane, the diacetate forms excellent crystals which melt at about 65–69°; but when recrystallized from aqueous methanol, the melting point is sharp and reproducible at 105–107°. Cumanin, recrystallized from ethyl acetate-hexane shows variable melting points of about 85–106°, but a sublimed sample had m.p. 132–124°. Whether those erratic melting points are caused by solvation or dimorphism is not known.

The structurally relevant NMR characteristics of cumanin-3-acetate are described in the discussion. The ultraviolet and i.r. spectra are unexceptional and in accord with the structure assigned.

Cumanin (VI) was isolated as the principal constituent in the fraction eluted by the more polar solvents. It had m.p. 110–112°, (reported[16] 83–85°; 120°) from ethyl acetate, and was identified by comparison with an authentic sample (m.p., TLC) by conversion to the diacetate, and by conversion to the acetonide.[16] The acetonide had m.p. 153–4° (reported 150–154°); its NMR spectrum agreed with that reported.[16]

Ambrosia psilostachya (No. 31568-APC) was processed in the manner described above. From 1·5 kg of dried plant was obtained 35 g of crude oily $CHCl_3$-extractable material, which upon chromatography over silica gel ($CHCl_3$, $CHCl_3$-methanol) yielded cumanin, cumanin-3-acetate, and cumanin diacetate. No coronopilin or parthenin were found.

Acknowledgements—This study was supported by a U.S. Public Health Service research grant GM-14240-02. Analyses are by Miss Heather King. We are grateful to Dr. J. Romo for exchange of information concerning the cumambrins and our compound, originally called artenovin, and for the comparison of our specimens with his.