iodine complex which slowly attacks olefin, but the presence of such a complex as well as its attack on olefin is difficult to consider. Another machanism which fits the rate equation is an attack of halogen on a π complex of olefin–peracid rapidly formed. The mechanism, however, is improbable because the rate of epoxidation with peracid is rather slow and no reasonable π complex is conceivable between peracid and olefin which may be attacked by halogen molecule to form the observed product.

The Structure of Psilostachyin C, a New Sesquiterpene Dilactone from *Ambrosia psilostachya* DC.

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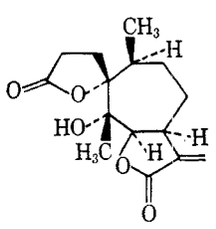
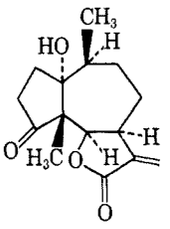
The structure of psilostachyin C, a new sesquiterpene dilactone from *Ambrosia psilostachya* DC., is shown to be **13**. Psilostachyin C was also isolated from *A. peruviana* Willd. and *A. deltoidea* (Torr.) Payne. Xantomicrol and damsin were also found in the last species.

We previously reported the structure of psilostachyin[5,6] (**1**), $C_{15}H_{20}O_5$, the major sesquiterpene lactone isolated from a Galveston Island, Texas, collection of the *Compositae* species, *Ambrosia psilostachya* DC. Two other sesquiterpene lactones, psilostachyin B, $C_{15}H_{18}O_4$, and psilostachyin C, $C_{15}H_{20}O_4$, were found in the Galveston Island material. Coronopilin[7,8] (**2**), parthenin[8] (**3**), and ambrosiol[8] (**4**) are the only other sesquiterpenes isolated from other collections of *A. psilostachya*. We now describe the structure determination of psilostachyin C and its synthesis from damsin (**12**), a sesquiterpene lactone of known structure.



1



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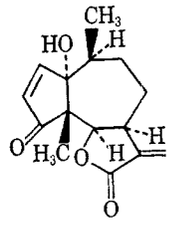

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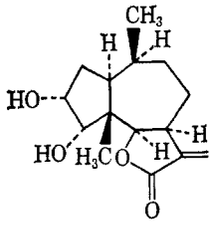
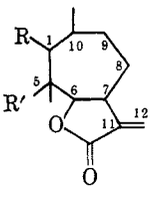


4

Psilostachyin C, $C_{15}H_{20}O_4$, mp 223–225°, $[\alpha]^{24}D$ −82 (*c* 0.6, $CHCl_3$), exhibited infrared (1775 and 1660 cm^{-1}) and ultraviolet (λ_{max} 210 mμ, ϵ 10,150) spectra typical for an α,β'-unsaturated γ-lactone of the type found in the other sesquiterpene lactones, **1–4**, already described from *A. psilostachya*. A second infrared carbonyl absorption (1730 cm^{-1}) corresponded to the absorption expected for either a δ-lactone group or a cyclopentanone ring. That psilostachyin C did not contain either a keto or a hydroxyl group was shown by the absence of ultraviolet absorption and Cotton effect characteristic of ketonic chromophores and our failure to observe signals typical of hydroxyl proton when the nmr spectrum of psilostachyin C was run in deuterated dimethyl sulfoxide.[6,9]

The nmr spectrum of psilostachyin C in $CDCl_3$ provided further evidence for an α,β'-unsaturated γ-lactone system of the type found in **1–4**; a doublet at 4.70[10] (J = 9 cps) was ascribed to the C_6 lactonic proton in **5** spin-coupled to one proton. Moreover, doublets (J = 3 cps) at 5.50 and 6.22 were typical for the two vinyl protons belonging to a methylene group attached to C_{11}. The nmr spectrum also displayed signals characteristic for one tertiary (1.27, singlet) and one secondary methyl group (1.02, doublet, J = 7 cps). Considering that **1** and psilostachyin C are found together in the same plant, the spectroscopic findings suggest partial formula A for psilostachyin C.



A

Evidence in addition to the infrared carbonyl absorption at 1730 cm^{-1} to support the presence of a δ-lactone group in psilostachyin C was provided by the following reactions. When psilostachyin C was treated with a methanolic solution of benzaldehyde and sodium methoxide, a crystalline derivative, **6** (Scheme I),

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(3) Florida State University. Paper V in a series "Constituents of *Ambrosia* and Related Species" from the Department of Chemistry, The Florida State University. Paper IV: W. Herz, H. Chikamatsu, and L. R. Tether, *J. Org. Chem.*, **31**, 1632 (1966).

(4) To whom inquiries should be addressed.

(5) H. E. Miller, H. B. Kagan, W. Renold, and T. J. Mabry, *Tetrahedron Letters*, **No. 38**, 3397 (1965).

(6) T. J. Mabry, H. E. Miller, H. B. Kagan, and W. Renold, *Tetrahedron*, in press.

(7) (a) W. Herz and G. Högenauer, *J. Org. Chem.*, **26**, 5011 (1961); (b) T. A. Geissman and R. J. Turley, *ibid.*, **29**, 2553 (1964).

(8) T. J. Mabry, W. Renold, H. E. Miller, and H. B. Kagan, *ibid.*, **31**, 681 (1966).

(9) O. L. Chapman and R. W. King, *J. Am. Chem. Soc.*, **86**, 1256 (1964).

(10) All chemical shift values are reported in parts per million (δ scale).

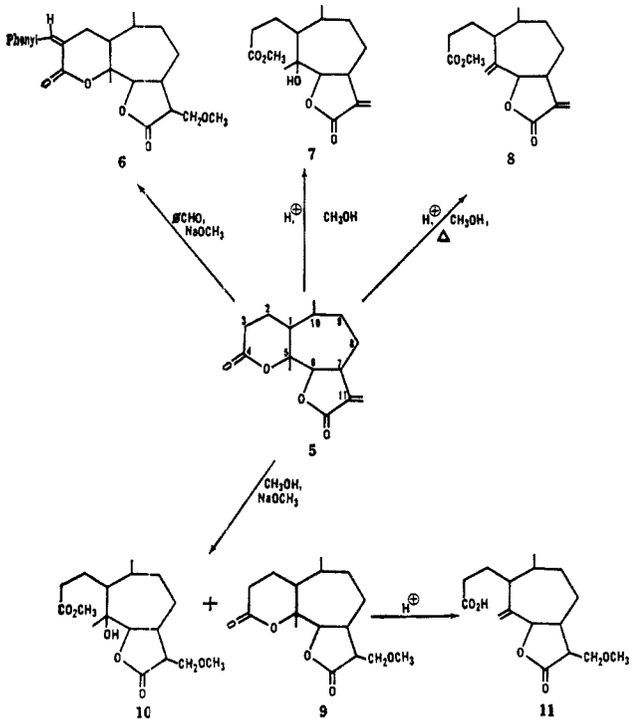
TABLE I

NMR SIGNALS OF PSILOSTACHYIN C DERIVATIVES[a]

Compd	H_6	H_7	C_{11}-CH_2	C_5-CH_2	C_5-Me	C_{10}-Me	Miscellaneous
5	4.70d(9)	3.5c	5.50d(4)		1.27	1.02d(7)	
			6.22d(4)				
6	4.57d(9)		3.62		1.45	1.10d(7)	7.82[b]
			3.67				7.40c[c]
							3.32[d]
7	4.45d(9)		5.40d(3)		1.05	0.86d(7)	3.55[d]
			6.12d(3)				
8	4.87d(9)	3.2c	5.55d(3)	4.85d(2)		0.72d(7)	3.62[d]
			6.22d(3)	5.31d(2)			
9	4.55d(9)		3.62d(4)		1.40	1.05d(7)	3.32[d]
10	4.50d(9)		3.68d(4)		1.26	1.03d(7)	3.39[d]
							3.70[e]
							4.45[f]
11	4.87d(9)		3.65	4.90d(2)		0.85d(7)	3.35[d]
				5.30d(2)			

[a] Spectra were determined in $CDCl_3$ on a Varian A-60 spectrometer. Values are given in parts per million relative to tetramethylsilane as an internal standard. Numbers in parentheses denote coupling constants in cycles per second. Signals in the first four columns correspond to one proton, signals in the next two to three protons, and signals in the last column as specified. Singlets are unmarked; multiplets are described as follows: d = doublet, c = complex signal whose center is given. [b] Vinyl proton. [c] Five aromatic protons. [d] Methoxyl. [e] Ester methoxyl. [f] Singlet for a tertiary hydroxyl group proton in deuterated dimethyl sulfoxide.

SCHEME I



$C_{23}H_{28}O_5$, mp 203–207°, was obtained. It was obvious from the nmr spectrum of **6** that not only had a benzylidene derivative of psilostachyin C been formed (five aromatic protons near 7.40 and a new vinylic proton at 7.8) but that Michael addition of methanol to the α,β'-unsaturated γ-lactone group had occurred as well (methoxyl singlet at 3.32; the absence of signals for the vinyl protons and new signals at 3.62 and 3.67 accounting for the C_{12} protons). The Michael reaction on the α,β'-unsaturated γ-lactone group was not unexpected, having been previously observed for a number of other sesquiterpene lactones.[6,11] The formation of the benzylidene derivative demonstrated the presence of a methylene group α to a carbonyl function in psilostachyin C, such as found in the δ-lactone ring of structure **5**.

That psilostachyin C did indeed contain a δ-lactone became clear when psilostachyin C was treated with acidic reagents. Psilostachyin C was transformed into an oily hydroxymethyl ester, to which we ascribe structure **7**, when **5** was allowed to stand in a methanolic solution of sulfuric acid. The nmr spectrum of the new derivative was wholly in accord with formula **7**: methoxyl signal at 3.55 and a singlet for a proton on a tertiary hydroxyl group at 4.75 (in deuterated dimethyl sulfoxide). The latter signal disappeared on addition of D_2O to the nmr tube. When psilostachyin C was refluxed with a methanolic solution of sulfuric acid, a different oily ester was obtained. The nmr spectrum of the new substance showed that the tertiary methyl group of psilostachyin C had disappeared and signals typical of two vinyl protons belonging to a new exocyclic methylene group had appeared (4.85, 5.32). The infrared spectrum supported structure **8** for this substance: 1760 (α,β'-unsaturated γ-lactone) and 1730 cm^{-1} (ester). The stability of the double bond exocyclic to the seven-membered ring under acidic conditions was of interest since it is known that 1-methylcycloheptene is more stable than methylenecycloheptane.[12]

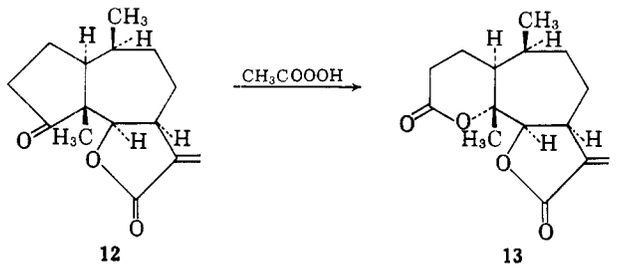
The following transformations permitted closer definition of the lactone functions. Treatment of psilostachyin C with methanolic sodium methoxide produced a mixture of the methanol Michael adduct of psilostachyin C, $C_{16}H_{24}O_5$ (**9**), mp 178–182°, and an oily methyl ester to which we assigned structure **10** on the basis of nmr data (Table I). When **9** was treated with acidic reagents, the dehydrated product, **11**, was formed. Compound **11** contained a stable double bond exocyclic to a seven-membered ring.

Final proof of structure and the stereochemical features of psilostachyin C were provided by the Baeyer–Villiger oxidation of damsin (**12**) to psilostachyin C. The synthetic material was identical in all respects with natural psilostachyin C. It is known that the Baeyer–

(11) (a) W. Herz, K. Ueda, and S. Inayama, *Tetrahedron*, **19**, 483 (1963); (b) W. Herz, Y. Kishida, and M. V. Lakshmikantham, *ibid.*, **20**, 979 (1964).

(12) Based on acid equilibration studies [A. C. Cope, D. Ambros, E. Ciganek, C. F. Howell, and Z. Jacura, *J. Am. Chem. Soc.*, **81**, 3153 (1959); *ibid.*, **82**, 1750 (1960)], and heats of hydrogenation data [R. B. Turner and R. H. Garner, *ibid.*, **80**, 1424 (1958)].

Villiger peracid oxidation provides a stereospecific cleavage of ketones.[13] Thus the absolute configuration of damsin at C_5 should be retained in the synthetic psilostachyin C. Damsin is known to be dihydroambrosin.[14] The stereochemical features shown in **12** rest on evidence previously presented for the steroechemical features of tetrahydroparthenin,[15] tetrahydroambrosin,[15] and coronopilin[7b] and have recently been confirmed by an X-ray analysis of 3-bromoambrosin.[16] Hence the absolute configuration of psilostachyin C can be formulated as **13**.



12 **13**

Psilostachyin C has been found to be identical with a compound isolated from *A. peruviana* Willd. and *A. deltoidea* (Torr.) Payne.[17] In the latter species we were also able to identify damsin and xanthomicrol (5,4′-dihydroxy-6,7,8-trimethoxyflavone) previously isolated from *Micromeria chamissonis*.[18] The peracid oxidation of damsin to psilostachyin C and the natural cooccurrence of damsin and psilostachyin C in *A. deltoidea* suggest the biogenesis of **13** from **12**.

Experimental Section[19]

Isolation of Psilostachyin C.—The isolation of psilostachyin C from *A. psilostachya* has been previously described.[6] An analytical sample melted at 225°: $[\alpha]^{24}D$ −82° (*c* 0.6, $CHCl_3$); circular dichroism[20] (ethanol): $\Delta\epsilon$ −2.6 at 209, +0.2 at 228, and −0.65 at 255 mμ; ultraviolet (methanol): λ_{max} 210 mμ (ε 10,150); infrared bands ($CHCl_3$): 1730 (δ-lactone), 1775 and 1660 cm^{-1} (α,β′-unsaturated γ-lactone); (Nujol): 1710 (δ-lactone), 1760 and 1660 cm^{-1} (α,β′-unsaturated γ-lactone).

Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.18; H, 7.58; O, 24.42; mol wt, 264.29. Found: C, 68.15; H, 7.45; O, 24.18. 264.29; mol wt (mass spectrum), 264.

Benzylidene Derivative–Michael Adduct (6).—Psilostachyin C (264 mg, 1 mmole) was dissolved in 5 ml of methanol containing 0.5 ml of benzaldehyde and 2 mmoles of sodium methoxide. After refluxing for 4 hr, the solution was acidified with dilute aqueous HCl and the product extracted with chloroform. The chloroform layer was washed with a dilute solution of sodium bicarbonate and then with water. When the chloroform was evaporated, 267 mg of an oil was obtained. Trituration of the oil with ether produced 82 mg of crystals, mp 138–158°, which was a 1:1 mixture of **6** and **9** by nmr analysis. Recrystallization of the mixture yielded **6**, mp 202–208°.

Anal. Calcd for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 72.18; H, 7.34.

When the same reaction was repeated at room temperature for 24 hr and using 160 mg of sodium hydroxide in place of the sodium methoxide, 30 mg of **6** was obtained.

Hydroxymethyl Ester (7).—Psilostachyin C (143 mg) was treated for 1 hr at room temperature with 4 ml of methanol containing 0.2 ml of concentrated sulfuric acid. After addition of water to the reaction solution, the product was extracted with chloroform. The chloroform layer, which was worked up in the usual manner, yielded 188 mg of an oil which contained mostly one substance by thin layer chromatography (tlc) on silica gel G (developing solution: chloroform–ethyl acetate, 2:1). The tlc plates indicated that the major constituent of the oil was a compound more polar than the starting material, **5**. The major component of the oil was assigned structure **7** primarily on the basis of the nmr data (Table I).

Dehydrated Methyl Ester (8).—When the experiment described above was repeated under reflux for 1 hr, 143 mg of an oil, **8**, pure by tlc was obtained: infrared bands (pure oil) at 1760 and 1660 (α,β′-unsaturated γ-lactone) and 1730 cm^{-1} (ester); the nmr data is tabulated in Table I.

If psilostachyin C was refluxed in formic acid or in a solvent (acetic acid or dioxane) containing a few drops of sulfuric acid, an acid analogous to the dehydrated methyl ester **8** was obtained: infrared bands ($CHCl_3$), broad hydroxyl signal between 3000–3500, 1710, and 1750 cm^{-1} (carboxyl and lactone groups).

Michael Adduct (9).—Psilostachyin C (539 mg) was refluxed for 1 hr in 5 ml of methanol containing 0.80 ml of a sodium methoxide solution (from 153 mg of sodium in 10 ml of methanol). The reaction solution, which was worked up in the manner described above for compound **6**, yielded 324 mg of crystalline material, mp 155–170°. The material was presumably a mixture of substances isomeric at C_{11}. An analytical sample was obtained by recrystallization from a chloroform–hexane solution: mp 178–182°; $[\alpha]^{24}D$ −50° (*c* 0.25, $CHCl_3$); infrared bands (Nujol) at 1760 and 1715 cm^{-1}.

Anal. Calcd for $C_{16}H_{24}O_5$: C, 64.85; H, 8.10. Found: C, 64.67; H, 8.06.

The mother liquor from the crystallization of **9** contained (by nmr) 15% of **9**, 15% of **5**, and 70% of a hydroxyl methyl ester (tertiary hydroxyl group: a singlet at 4.45 in deuterated dimethyl sulfoxide which disappeared on addition of D_2O; for other nmr signals see Table I). We assigned structure **10** to the new substance: infrared bands (oil) at 3450 and a broad signal between 1725–1752 cm^{-1}.

Dehydration of 9.—The Michael adduct **9** (110 mg) was refluxed for 1 hr in 1.6 ml of dioxane containing 0.1 ml of concentrated sulfuric acid. An ether extraction of the reaction solution yielded 100 mg of an oil to which we assigned structure **11** on the basis of nmr data (Table I) and infrared bands (pure oil): broad signals at 3000–3500 and 1700–1755 cm^{-1}.

Synthesis of Psilostachyin C from Damsin (12).—Damsin (518 mg) was allowed to stand overnight at room temperature with 3 ml of 40% peracetic acid. The formation of psilostachyin C could be followed by tlc (silica gel G; chloroform–ethyl acetate: 3:2) since psilostachyin C is more polar than **12**. After adding water to the reaction solution, the product was extracted with chloroform. The chloroform layer was washed with a sodium bicarbonate solution and then with water. Evaporation of the chloroform afforded oily material. The oil was washed with hexane and then ether. Evaporation of the ether yielded 74 mg of almost pure damsin. The remaining oily material (386 mg) was chromatographed on a column of silica gel. Elution of the column with chloroform gave first 74 mg of damsin, then 36 mg of a mixture of damsin and psilostachyin C, and finally 179 mg of psilostachyin C, mp 220° (no depression with an authentic sample). The synthetic material was identical with natural psilostachyin C by ultraviolet, infrared, and nmr spectra and by tlc.

Extraction of *A. peruviana* Willd.—Dried leaves and flowerheads of *A. peruviana* Willd., wt 1.5 kg, collected in July 1963, in the vicinity of Mayaguez, Puerto Rico, were extracted with chloroform and worked up in the usual way.[7] The crude gum, wt 12 g, was recrystallized directly from acetone–dry ether. This furnished 1.89 g of slightly impure psilostachyin C. An additional recrystallization from chloroform–hexane raised the mp to 232° (with previous shrinking above 220°), $[\alpha]^{21}D$ −80° (*c* 0.9, $CHCl_3$), identical in all respects (infrared and nmr spectrum, tlc, mixture melting point) with psilostachyin C from *A. psilostachya*.

(13) (a) R. B. Turner, *J. Am. Chem. Soc.*, **72**, 579 (1950); (b) K. Mislow and J. Brenner, *ibid.*, **75**, 2318 (1953).

(14) M. Suchy, V. Herout, and F. Sorm, *Collection Czech. Chem. Commun.*, **28**, 2257 (1963).

(15) W. Herz, H. Watanabe, M. Miyazaki, and K. Kishida, *J. Am. Chem. Soc.*, **84**, 2601 (1962).

(16) M. T. Emerson, C. N. Caughlan, and W. Herz, to be published.

(17) For nomenclature of this species, previously known as *Franseria deltoidea* Torr., see W. W. Payne, *J. Arnold Arboretum*, **45**, 401 (1964).

(18) G. H. Stout and V. F. Stout, *Tetrahedron*, **14**, 296 (1961). We wish to thank Professor Stout for an authentic sample of xanthomicrol which made the comparison possible.

(19) Melting points are uncorrected. Analyses were determined by Dr. Alfred Bernhardt, Max-Planck Institut für Kohlenforschung, Mülheim, West Germany.

(20) We thank Dr. M. Legrand, Roussel UCLAF, Paris, for this determination.

Chromatography of the material from the mother liquors (wt 10 g) furnished small amounts of unidentified sesquiterpene lactones which we plan to investigate when more plant material becomes available.

Extraction of *A. deltoidea* (Torr.) Payne.—Leaves and flowerheads collected by Mr. R. J. Barr on April 14, 1961, in Pinal County, Arizona (RJ Barr No. 61-442), wt 740 g, were extracted with chloroform and worked up in the usual way.[7] The residual gum, wt 64 g, was taken up in chloroform–benzene (1:1) and chromatographed over 660 g of silicic acid (Mallinckrodt 100–200 mesh). Fractions 1–3 (benzene–chloroform, 1:1) eluted 0.1 g of unidentified material, fractions 4–7 (benzene–chloroform, 1:3), wt 12.8 g, containing damsin (tlc). Rechromatography and recrystallization furnished 2.4 g of pure damsin, mp 100°, identified by comparison (infrared, nmr, tlc, melting point) with authentic material.[21] Fraction 8 (benzene–chloroform 1:3) was a complex mixture, wt 2.1 g; fractions 9–10 (chloroform), wt 4.4 g, contained two compounds (tlc) and were, therefore, rechromatographed. The less polar substance, mp 230°, wt 0.15 g, was identical with psilostachyin C (infrared and nmr spectrum, tlc); the more polar substance, wt 1.3 g, could not be obtained in pure form, but appeared to be a carboxylic acid. More of this material was found in the later chloroform eluates (fractions 11–14, wt 13.3 g) which were reserved for future purification and investigation. Chloroform–methanol (three fractions, 97:3) eluted 2.8 g of yellow material which was recrystallized from methanol: yield 1.1 g; mp 228–230°. The infrared spectrum of this substance suggested that it was a flavone. The nmr spectrum (trifluoroacetic acid) exhibited doublets at 8.28 d (J = 8.5, two protons) and 7.30 (J = 8.5, two protons) typical of the A_2B_2 system of ring B of 4′-substituted flavones, a singlet at 7.40 (probably H_3), and three methyl singlets at 4.40, 4.25, and 4.16 ppm. Color reactions and ultraviolet spectrum indicated the presence of a free hydroxyl group at C_5. This was confirmed by methylation with diazomethane. The resulting monohydroxytetramethoxyflavone, mp 169–172°, had nmr signals (deuteriochloroform) at 12.3 (C_5-hydroxyl), 7.7 d and 6.84 d (A_2B_2 system), 6.4 (H_3), and four methoxyls at 3.75–3.95 ppm. The diacetate, prepared with acetic anhydride–pyridine, melted at 128°. The identity with xanthomicrol (5,4′-dihydroxy-6,7,8-trimethoxyflavone, lit.[18] mp 227–230°, lit.[18] mp of monomethyl ether 180–181°, lit.[18] mp of diacetate 126.5–128.5°) suggested by these experiments was confirmed by direct comparison (mixture melting point and infrared spectra) with an authentic sample of xanthomicrol supplied by Professor Stout.

(21) W. Herz and Y. Sumi, *J. Org. Chem.*, **29**, 3438 (1964).

Acknowledgments.—T. J. M. thanks the Robert A. Welch Foundation (Grant F-130) for financial support. H. E. M. thanks the National Insitutes of Health (Grant 5Tl GM-789) for a predoctoral fellowship. H. B. K. acknowledges a NATO travel grant (1965). The work at The Florida State University was supported by a grant from the U. S. Public Health Service (GM-05814). We also wish to thank The Florida State University Research Council for a grant to help defray the cost of plant collections.

Notes

Constituents of *Ambrosia ilicifolia* (Gray) Payne[1,2]

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Received December 22, 1965

Because of the debate about the position and relative status of *Ambrosia* and its allies within the family *Compositae*,[3] we have undertaken a phytochemical study of these genera.[4] In the present paper we report our work on the constituents of *A. ilicifolia* (Gray) Payne, a species native to the Sonora desert.[5] Work on other species is in progress.

Extraction of *A. ilicifolia* (Gray) Payne with chloroform in the usual manner[3] furnished in approximately 0.17% yield an acidic substance $C_{15}H_{24}O_3$, mp 176–177°, $[\alpha]^{31}D$ −35.1°, which we have named ilicic acid.

The infrared spectrum of ilicic acid (**1a**), in addition to acid absorption at 1690 cm^{-1}, exhibited bands at 1620 and 914 cm^{-1} characteristic of an exocyclic methylene group which was probably conjugated with the carboxyl (λ_{max} 206 mμ, ϵ 9100). This was supported by the infrared spectrum of the methyl ester (**1b**) which had bands at 1715 (conjugated ester), 1625 and 912 cm^{-1} (exocyclic methylene group), as well as absorption establishing the presence of a hydroxyl group. Hence all three oxygen atoms were accounted for and since catalytic hydrogenation resulted in the uptake of only 1 mole of hydrogen, ilicic acid had to be bicyclic.

The nmr spectra of **1a** and **1b** exhibited signals indicating the presence of two tertiary methyl groups (at 1.10 and 0.94 ppm in **1a**, at 1.07 and 0.86 in **1b**). Noticeable also were two slightly split resonances characteristic of the exocyclic methylene group whose

(1) Paper IV in a series "Constituents of *Ambrosia* and Related Species." Previous paper: W. Herz and Y. Sumi, *J. Org. Chem.*, **29**, 3438 (1964).

(2) Supported in part by a grant from the U. S. Public Health Service (GM-05814). We also wish to acknowledge a grant from the Florida State University Research Council to defray the cost of plant collections.

(3) For pertinent references, see ref 1 and W. Herz and G. Högenauer, *J. Org. Chem.*, **26**, 5011 (1961).

(4) For our most recent results on *Iva* species, see W. Herz, A. Romo de Vivar, and M. V. Lakshmikantham, *ibid.*, **30**, 118 (1965).

(5) This species has previously been known as *Franseria ilicifolia* Gray. Compelling arguments have recently been advanced for the congeneric nature of *Ambrosia* ("true ragweeds") and *Franseria* ("false ragweeds")[6] and we have, therefore, adopted the nomenclature proposed by Payne.[6] *Franseria* species, *sensu stricto*, which have been investigated previously include *A. dumosa* (Gray) Payne (*F. dumosa* Gray) which yields the pseudoguaianolide coronopilin,[3,7] *A. chamissonis* (Less.) Greene (*F. chamissonis* Less.) which yields the germacranolide chamissonin,[8] and *A. deltoidea* (Torr.) Payne (*F. deltoidea* Torr.) which yields damsin and the modified pseudoguaianolide psilostachyin C.[9]

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