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]

WERNER HERZ, HIROAKI CHIKAMATSU, AND L. R. TETHER

Department of Chemistry, The Florida State University, Tallahassee, Florida 32306

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]

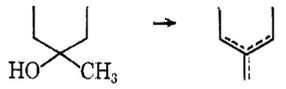
(6) W. W. Payne, *J. Arnold Arbor.*, **45**, 401 (1964).

(7) T. A. Geissman and R. J. Turley, *J. Org. Chem.*, **29**, 2553 (1964).

(8) R. J. Turley, *Dissertation Abstr.*, **25**, 6245 (1965).

(9) H. E. Miller, H. B. Kagan, W. Renold, and T. J.Mabry, *Tetrahedron Letters*, 3397 (1965); 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).

chemical shift (6.20 and 5.65 ppm in **1a**, 6.09 and 5.51 in **1b**) was indicative of conjugation. These disappeared on conversion of **1a**, by treatment with excess diazomethane, to the crystalline pyrazoline methyl ester **4a**, and on catalytic reduction to oily **2a**. Because of the absence of other low-field absorption the hydroxyl group had to be tertiary. Moreover, since treatment of **3a** with methanesulfonyl chloride resulted in formation of an olefin mixture of type B (nmr spectrum), partial structure A was present in ilicic acid.

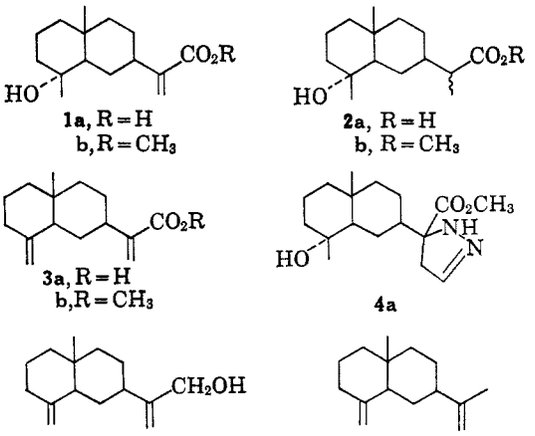


A B

A minor constituent of *A. ilicifolia* was a second acid $C_{15}H_{22}O_2$, mp 88–89°, $[\alpha]^{31}D$ +29.1°, whose infrared spectrum (bands at 1690, 1640, and 1620 cm^{-1}, methyl ester absorbs at 1720, 1640, and 1620 cm^{-1}) indicated the presence of two double bonds. The nmr spectrum of this substance (methyl singlet at 0.77, two pairs of vinyl proton singlets at 6.40 and 5.73 and at 4.76 and 4.46 ppm, methyl ester signals at 6.17, 5.57, 4.72, 4.42, 3.75(methoxyl), and 0.74 ppm) suggested that the two acids were intimately related, with the minor component representing a dehydration product of ilicic acid.

At this point there appeared a report on the isolation, from the acid fraction of costus oil, of the new sesquiterpene acid, costic acid, for which formula **3a** was established by correlation with costol (**5**) and β-selinene (**6**). The physical properties of costic acid, mp 87–88°, $[\alpha]^{26}D$ +23.42°, were so similar to those of the minor acid from *A. ilicifolia* that a direct comparison was called for. Although an authentic specimen of costic acid was not available, comparison of a sample of methyl costate[11] with the methyl ester of the minor acid by nmr, infrared, and glc established their complete identity. That ilicic acid must be **1a** (Scheme I) was then demonstrated in the following manner. Low-temperature dehydration of **1b** with phosphorus oxychloride–pyridine resulted in a mixture containing *ca.* 70% of **3b** and smaller amounts of the Δ^3 and $\Delta^{4,5}$ isomers. Hydrolysis of the mixture and fractional crystallization of the crude product furnished costic acid, identical with the minor constituent. Because of the predominance of **3b**, the hydroxyl group of ilicic acid must be equatorial and α.

SCHEME I



Experimental Section[11]

Extraction of *A. ilicifolia* (Gray) Payne.—The results of a large-scale extraction of dried above-ground material collected by Mr. R. J. Barr on May 19, 1965, at 600-ft elevation near Telegraph Pass in the Gila Mountains, Yuma County, Ariz. (Barr No. 65-214) essentially paralleled those obtained from a preliminary run using 1.4 kg of material collected in the same locality on March 26, 1963 (Barr No. 63-97). Extraction of a 13-lb portion with chloroform and work-up in the usual manner[3] gave 92 g of crude gum which was taken up in benzene. The benzene layer was extracted with 10% sodium carbonate solution, the latter was acidified with dilute sulfuric acid, and the acid material was extracted with benzene. The benzene extract was washed, dried, and concentrated. On cooling there precipitated crude ilicic acid, wt 10.13 g. The mother liquor was concentrated *in vacuo* and the residue, 14.7 g, taken up in 150 ml of benzene–chloroform (1:1) and chromatographed over 110 g of silicic acid, eluent benzene–chloroform (1:1), 75-ml fractions. Fractions 1–3 eluted 0.9 g of semicrystalline material (mainly costic acid) and fractions 5–9 eluted 2.8 g of crude ilicic acid; total yield 12.93 g (0.22%). Recrystallization from ethanol–water gave pure ilicic acid: wt 10.1 g (0.17%); mp 176–177°; $[\alpha]^{31}D$ −35.1° (*c* 1.372); infrared bands (Nujol) at 3500 (chloroform), 3500–2300 (br band, carboxyl), 1690, 1620, and 914 cm^{-1}; nmr signals (deuterioacetone) at 6.20 d (1.5) and 5.65 d (2, H_{13}), 1.10 (C_4-methyl), and 0.94 ppm (C_{10}-methyl); λ_{max} 206 mμ (ε 9100).

Anal. Calcd for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59; O, 19.02. Found: C, 71.44; H, 9.59; O, 19.32.

A solution of 1.454 g of ilicic acid in 100 ml of ether was mixed with the calculated amount of diazomethane in ether. The solution was allowed to stand and evaporated at reduced pressure, and the oil **1b** was chromatographed over silicic acid: wt 1.5 g; $n^{27}D$ 1.5084; $[\alpha]^{29}D$ −35.8° (*c* 1.5); infrared bands at 3450, 1710, and 1620 cm^{-1}; nmr signals at 6.09 d (1.5) and 5.51 t (1, H_{13}), 3.70 (methoxyl), 1.07 (C_4-methyl), and 0.86 ppm (C_{10}-methyl).

Anal. Calcd for $C_{16}H_{26}O_3$: C, 72.14; H, 9.84; O, 18.02. Found: C, 71.95; H, 9.97; O, 18.35.

Treatment of **1a** with excess diazomethane gave solid material, **4a**, which was recrystallized from benzene–petroleum ether: mp 120°; infrared bands at 3500 (–OH), 1725 (ester), and 1610 cm^{-1} (C=); nmr signals at 4.56 t (8, –C*H*=), 3.76 (methoxyl), 1.07 (C_4-methyl), and 0.85 ppm (C_{10}-methyl).

Anal. Calcd for $C_{17}H_{28}N_2O_3$: C, 66.20; H, 9.15; N, 9.08. Found: C, 66.02; H, 9.00; N, 9.33.

Catalytic hydrogenation of 0.331 g of ilicic acid in 50 ml of ethanol with 40 mg of prereduced platinum oxide required 29.9 ml of hydrogen at STP; calculated for one double bond, 29.7 ml. The product could not be induced to crystallize, but was homogeneous on thin layer chromatography: nmr bands at 1.16 d (7, C_{11}-methyl), 1.10 (C_4-methyl), and 0.86 ppm (C_{10}-methyl).

The semicrystalline material obtained from the first fractions of the chromatogram of the ilicic acid mother liquors was recrystallized from ethanol–water; yield 0.6 g (0.01%) of slightly impure costic acid. The material was dissolved in petroleum ether (bp 30–60°) and chromatographed over 8 g of silicic acid. Petroleum ether–benzene (1:1) eluted 0.29 g of costic acid, mp 84–85°, whose melting point was raised to 88–89° after an additional recrystallization from ethanol–water: $[\alpha]^{31}D$ +29.1° (*c* 1.057); infrared bands at 1690, 1640, and 1620 cm^{-1}; nmr signals at 6.40, 5.73 (H_{13}), 4.76 and 4.46 (H_{14}), and 0.77 ppm (C_{10}-methyl).

Anal. Calcd for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46; O, 13.66. Found: C, 77.44; H, 9.13; O, 13.53.

Treatment of 0.17 g of costic acid in 10 ml of ether with the calculated amount of diazomethane gave, after the usual work-up,

(10) A. S. Bawdekar and G. R. Kelkar, *Tetrahedron*, **21**, 1521 (1965).

(11) We wish to thank Dr. G. R. Kelkar, National Chemical Laboratory, Poona, for supplying us with the material.

(12) Melting points and boiling points are uncorrected. Analyses were determined by Dr. F. Pascher, Bonn, Germany. Infrared spectra were run in chloroform, ultraviolet spectra in 95% ethanol, rotations in chloroform, and nmr spectra in deuteriochloroform, unless otherwise specified on an A-60 spectrometer with tetramethylsilane serving as internal standard. Signals are reported in parts per million. Gas–liquid partition chromatograms were run on an F and M Model 500 instrument using a 4-ft FFAP column held at 200°, carrier gas helium at 33 cc/min.

an oil which was purified by chromatography over acid-washed alumina: $n^{29}D$ 1.502; $[\alpha]^{31}D$ +24° (*c* 1.0); infrared bands at 1720, 1640, and 1620 cm^{-1}; nmr signals at 6.17 d (1.5) and 5.57 t (1, H_{13}), 4.72 d (2, 1.5) and 4.42 t (2, 2, H_{14}), 3.75 (methoxyl), and 0.74 ppm (C_{10}-methyl). These figures correspond closely to those reported[10] for methyl costate. Direct comparison of this material with an authentic sample of methyl costate established identity of infrared and nmr spectra. The two specimens were indistinguishable on tlc and had identical retention times on glpc.

Conversion of Ilicic Acid to Costic Acid.—To a solution of 1.50 g of methyl ilicate in 23 ml of pyridine was added dropwise, with cooling, 4.6 ml of phosphorus oxychloride. After 12 hr at room temperature, the mixture was poured into ice water and extracted with ether. The ether extracts were washed thoroughly with water, dilute acid, and water and were dried and evaporated *in vacuo*. The residue was chromatographed over acid-washed alumina and the product eluted with petroleum ether: wt 0.96 g, $n^{29}D$ 1.5055, $[\alpha]^{31}D$ +25.8° (*c* 2.145). Although the thin layer chromatogram of this material showed only one spot corresponding to that of authentic methyl costate, the nmr spectrum exhibited additional methyl singlets at 1.07 and 0.82 ppm corresponding to the C_{10}-methyl of the $\Delta^{4,5}$ and Δ^{3} isomers, respectively, an additional vinyl methyl singlet at 1.63 ppm ($\Delta^{4,5}$ and Δ^{3} isomers), and a vinyl proton multiplet at 5.33 ppm (Δ^{3} isomer). The intensity of these signals indicated a composition of *ca.* 70% methyl costate, 15% Δ^{3}, and 15% $\Delta^{4,5}$-isomer. Glc gave a main peak (*ca.* 75%) corresponding to methyl costate, with the minor peaks of the other two isomers almost superimposed.

The mixture of esters, wt 0.9 g, was hydrolyzed by refluxing with 25 ml of 6% methanolic potassium hydroxide for 1.5 hr. The mixture was concentrated at reduced pressure, diluted with water, acidified, and extracted with ether. The washed and dried ether extract was evaporated and the residual oil, wt 0.87 g, was chromatographed over 8 g of silicic acid. Benzene–petroleum ether (3:1) eluted semicrystalline material, wt 0.49 g, which after several recrystallizations from ethanol–water, melted at 88.5–89.5°, $[\alpha]^{30}D$ +28° (*c* 0.665), melting point undepressed on admixture of costic acid, infrared spectra superimposable.

The Configuration of (+)-S-(1-Propenyl)-L-Cysteine S-Oxide from *Allium cepa*

J. F. CARSON,[1] R. E. LUNDIN,[1] AND THOMAS M. LUKES[2]

Western Regional Research Laboratory,[1] Albany, California 94710, and Gentry Division, Consolidated Foods,[2] Gilroy, California

Received November 29, 1965

Virtanen and Spåre[3] isolated from *Allium cepa* (onion) an amino acid which was shown to be the precursor of the lachrymatory property of the vegetable. The structure of this compound was established as (+)-S-(1-propenyl)-L-cysteine S-oxide[4] but the configuration of the olefinic double bond was left undecided. This report presents evidence based on nuclear magnetic resonance spectra that the compound is the *trans* isomer.

Our compound was isolated from commercial dehydrated onions and was shown to be identical with Virtanen's amino acid by elemental analysis, specific rotation, susceptibility to onion enzyme or to *Albizzia lophanta* C–S-lyase[5] to produce onion odor and lachrymatory properties, and particularly by the observation that the compound cyclizes in dilute ammonia to produce cycloalliin (3-methyl-1,4-thiazane-5-carboxylic acid S-oxide) in high yield. In the infrared, the compound shows strong absorption in the sulfoxide[6] region at 1025 and 1037 and also at 967 cm^{-1} suggesting a *trans* double bond.

The 60-Mc proton magnetic resonance (pmr) spectrum of the amino acid in deuterium oxide confirmed a *trans* configuration for the double bond. The ABX_3 multiplet at τ 3.4 arising from the olefinic protons clearly shows a 15–16 cps *trans* coupling between these protons, although an exact analysis of this complex multiplet was not attempted. To further strengthen this assignment a spectrum was also obtained at 100 Mc/sec. The additional separation of the two protons at the higher frequency makes the interpretation of the *trans* coupling unequivocal.

Two crystalline derivatives, the N-2,4-dinitrophenyl- and the N-2,4,6-trinitrophenylpropenylcysteine S-oxides were prepared. The pmr spectra of these compounds in deuterated dimethyl sulfoxide also showed a *trans* coupling of the olefinic protons (J = 16 cps).

Experimental Section

The pmr spectra were obtained on Varian A-60 and HR-100 spectrophotometers. Tetramethylsilane was the internal standard with deuterated dimethyl sulfoxide and sodium 3-(trimethylsilyl)-1-propanesulfonate was the internal standard with deuterium oxide. Infrared spectra were obtained with a Perkin-Elmer Model 237 instrument.

Isolation of (+)-S-(1-Propenyl)-L-cysteine S-Oxide.—Commercially dehydrated white onion powder (2 kg) was slowly added to 16 l. of boiling distilled water with vigorous stirring. The mixture was then allowed to stand for 4 min and 5 kg of ice was added. The slurry was filtered and the filtrate was adjusted to pH 4 with acetic acid. Approximately 20% of the juice was poured through a column of Dowex 50-X4 (H^+) (7 × 30 cm) and the column then eluted with 0.1 *M* sodium acetate adjusted to pH 6.5 with acetic acid. As soon as ninhydrin-positive material appeared in the eluate, 250-ml aliquots were collected. These were tested for the presence of the propenylcysteine sulfoxide by adding onion enzyme and organoleptically detecting the lachrymator. The amino acid emerges with the acidic amino acids and is completely eluted by the time a characteristic brown pigment emerges from the column. The remaining onion juice was treated with ion exchanger in the same manner and the combined fractions containing the amino acid precursor were passed through a second column of Dowex 50-X4 (H^+) (2.5 × 50 cm), and the amino acids were eluted with 0.05 *N* sodium hydroxide at 30 ml/hr and collected in 100-ml aliquots.

The fractions which contained the precursor were combined and passed through a column of Dowex 2-X8 (2 × 15 cm) in the acetate form to remove acidic amino acids. The lachrymatory precursor was not absorbed. Finally, the eluate was absorbed in a column of Dowex 50-X4 (2.5 × 50 cm); the amino acids were eluted with 0.05 *N* ammonium hydroxide. Fractions (50 ml) were collected and those that were chromatographically homogeneous on paper were combined, adjusted to pH 6.5 with acetic acid, and taken to dryness *in vacuo*. Several recrystallizations of the solid residue from aqueous acetone yielded the pure amino acid in a yield of 3 g from 2 kg of onion powder.

The compound decomposed sharply at 153° (lit.[3] dec pt 146–148°) and yielded one spot on paper chromatography with butanol–acetic acid–water (63:10:27) at 25°; relative R_f with respect to alanine 1.45; $[\alpha]^{26}D$ +74.9° (*c* 6.2, water) (lit.[3] value +74°).

Anal. Calcd for $C_6H_{11}NO_3S$: C, 40.66; H, 6.39; N, 7.90. Found: C, 40.8; H, 6.39; N, 7.84.

(1) A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

(2) Inquiries may be sent to: California State Polytechnic College, Food Processing Department, San Luis Obispo, Calif.

(3) A. I. Virtanen and C. G. Spåre, *Suomen Kemistilehti*, **B34**, 72 (1961); **B35**, 28 (1962).

(4) C. G. Spåre and A. I. Virtanen, *Acta Chem. Scand.*, **17**, 641 (1963).

(5) S. Schwimmer and A. Kjaer, *Biochim. Biophys. Acta*, **42**, 316 (1960).

(6) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1958, pp 49–51, 357–359.