(14) The cholesterol synthesis was tested *in vitro* by a rat liver homogenate preparation. A pooled liver homogenate was incubated simultaneously with acetate-^{14}C and mevalonate-^{3}H in the presence of different concentrations of 22-oxocholesterol oxime and the incorporation into nonsaponifiable lipids and cholesterol was measured.
(15) Other compounds in this series have not been assayed as yet.
(16) For procedure compare R. C. Nickolson and M. Gut, *J. Org. Chem.*, **37**, 2119 (1972).

New Monoterpenes from *Artemisia filifolia* (Torrey). Structure, Synthesis, Rearrangements, and Biosynthesis[1a]

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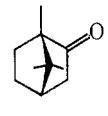
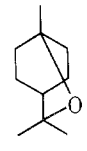
The steam distillate of the leaves and stems of sand sage [*Artemisia filifolia* (Torrey)] contains two new monoterpene lactones, filifolide A and filifolide B. These have been characterized as 1(*R*),5(*S*)-(−)-5-hydroxy-2,2,4-trimethylcyclohex-3-ene-1-carboxylic acid γ-lactone (**8**) and 1(*R*),3(*S*)-(+)-3-hydroxy-2,2,4-trimethylcyclohex-4-ene-1-carboxylic acid γ-lactone (**9**). Other major constituents of the distillate are the cyclobutanone (−)-filifolone, (−)-camphor, 1,8-cineole, and isophorone. Minor constituents are piperitenone, borneol, (−)-verbenone, and 3,3,5-trimethylcyclohex-2-ene-1,4-dione (**13**). The chloroform extract of the plant contains the monoterpene 1(*R*)-(+)-5-keto-2,2,4-trimethylcyclohex-3-ene-1-carboxylic acid (**14**), the flavone acacetin, and the sesquiterpene lactone colartin. In addition to the synthesis of lactones **8** and **9**, another new monoterpene lactone was prepared, 2-hydroxy-α,α,3-trimethylcyclopent-3-ene-1-acetic acid γ-lactone (**2**).

Sand sage [*Artemisia filifolia* (Torrey)] has been used by Hopi Indians and early white settlers as a medicinal plant.[1b] It grows at elevations ranging from 4000 to 6000 ft in the state of Arizona. The strong "cough medicine" fragrance of the plant, particularly after a rainfall, prompted us to investigate the steam distillate of the plant.

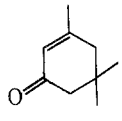
Preliminary studies[2] showed a relatively high (1% wet weight) percentage of steam volatile oil. The major constituents were separated by gas–liquid chromatography and identified as (−)-camphor, 1,8-cineole, and isophorone. The structure of the fourth constituent was subsequently shown[3] to be the cyclobutanone (−)-filifolone.



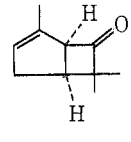
(−)-camphor



1,8-cineole

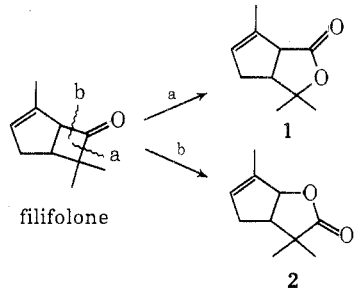


isophorone



(−)-filifolone

A fifth fraction appeared to be a 1:1 mixture of two monoterpene lactones (filifolide A and filifolide B), both having the molecular formula $C_{10}H_{14}O_2$. Catalytic hydrogenation of the lactone mixture gives a neutral and an acid fraction, indicating an allylic lactone system.[4] Structures **1** and **2** were originally proposed for the two lactones on the assumption that these could have arisen by a simple Baeyer–Villiger oxidation of filifolone. The present report is chiefly concerned with the structures of filifolides A and B.

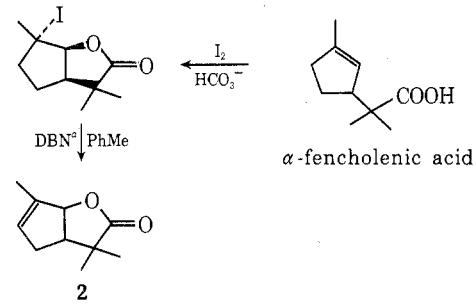


filifolone

1

2

Scheme I
The Synthesis of Lactone 2

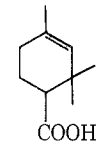


[a] DBN = 1,5-diazabicyclo[4.3.0]non-5-ene.

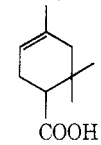
Results

Lactones. Compound **1** (carvenolide) has been reported by Wallach more than 70 years ago.[5] Its structure was recently confirmed[6] and its nmr spectrum[6] was shown to be different from those of filifolides A and B. Compound **2** had not been previously reported; so its synthesis was carried out, as shown in Scheme I. The spectral data for lactone **2** did not coincide with either filifolide A or B from *A. filifolia;* hence neither structure **1** or **2** was tenable.

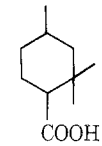
A careful reexamination of the hydrogenation reaction gave us the first clue to the structures of filifolides A and B. Surprisingly, both lactones underwent hydrogenolysis to yield two unsaturated carboxylic acids, both $C_{10}H_{16}O_2$, which on further hydrogenation gave the single acid, $C_{10}H_{18}O_2$. Their respective structures were deduced from nmr spectra reported[7] for **3** and **4** and from the hydrogenation product **5**.



3

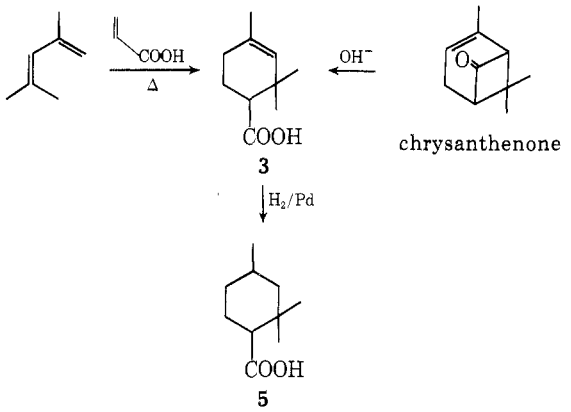


4

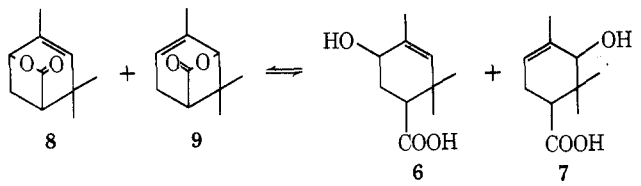


5

Compounds 3 and 5 were prepared by two independent routes[7,8] and shown to be identical with the hydrogenation products of filifolides A and B. Thus, 3 and 4 should represent the basic carbocyclic structures of the lactones, less the oxygen function.



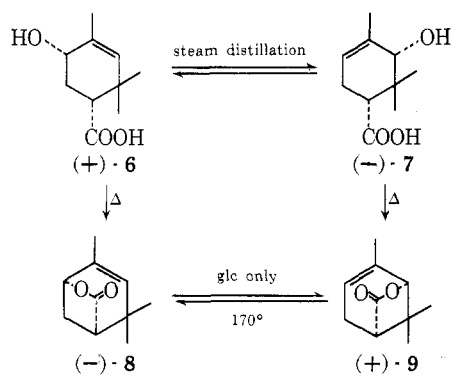
Alkaline hydrolysis of the lactone mixture gave two acids, which were separated by fractional crystallization. One of these was identified as 5-hydroxy-2,2,4-trimethylcyclohex-3-ene-1-carboxylic acid (6)[9] and the other acid was assigned the structure 7, based on spectral evidence. When 6 and 7 were heated separately, they gave lactones A and B, respectively. Therefore, structures 8 and 9 could be tentatively assigned to the filifolides A and B.



The final confirmation of the lactone structures was obtained by independent synthesis. Lactone 8 was prepared by two methods, the first of which established the absolute configuration of the enantiomer in the plant, *i.e.*, (−)-8.

Lactone B (9) proved more difficult to synthesize, because epoxidation of one of the intermediates (10) was not stereospecific and resulted in a mixture of epoxides (11 and 12), as in Scheme II, method 3.

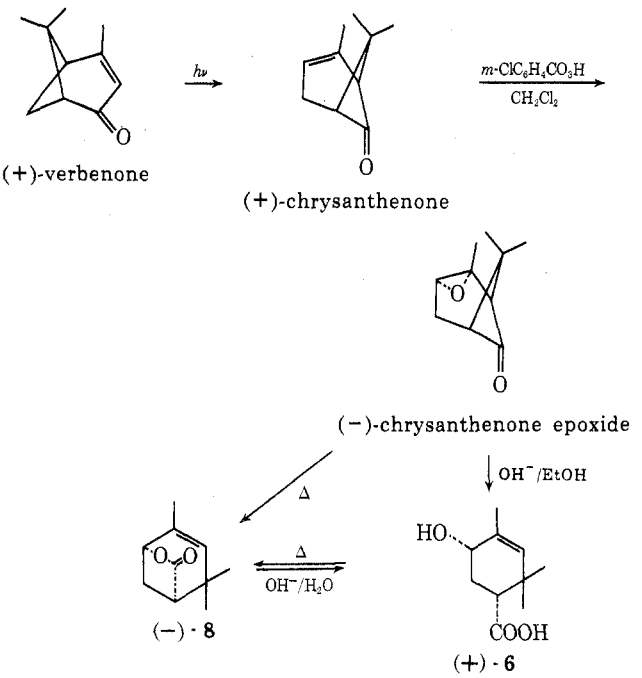
Lactones (−)-8 and (+)-9 occur in the steam distillate of the plant material in approximate ratio of 5:1, respectively. However, if the steam distillate is subjected to gas–liquid chromatography at 170°, the ratio is invariably 1:1. If filifolide A is injected into the glc apparatus, a 1:1 mixture occurs. However, if A is steam distilled, no interconversion takes place. On the other hand, hydroxy acids 6 and 7 are readily interconverted by steam distillation. This behavior is summarized below.



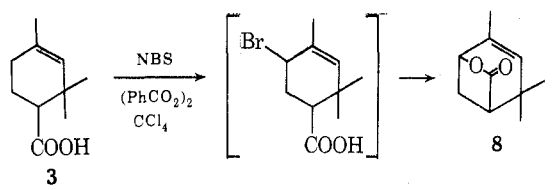
The interconversion of 8 and 9 allowed the absolute configuration of lactone 9 from *A. filifolia* to be determined, since the absolute configuration of (−)-8 was known. The 1:1 (approximately) mixture of lactones 8 and 9 obtained upon preparative glc of (−)-8 was found to have a specific rotation approximately equal to the sum of the specific rotations of (−)-8 and (+)-9 found in *A. filifolia*. This demonstrated that the thermolysis product of (−)-8 is (+)-9, that very little racemization of 8 or 9 was occurring during glc, and that (+)-9 from *A. filifolia* has the absolute configuration shown above.

Scheme II

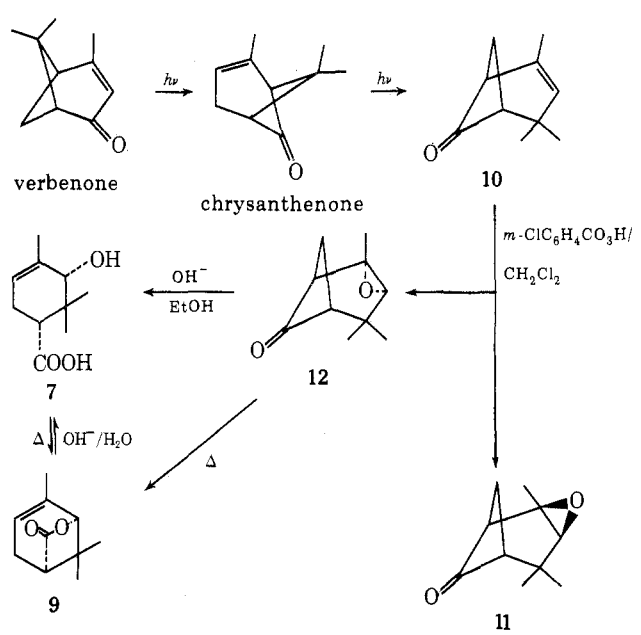
Method 1



Method 2

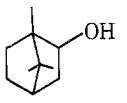


Method 3

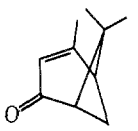


Neither **6** nor **7** were found in the chloroform extract of the plant, thus excluding the possibility that lactones **8** and **9** could be artifacts of the steam distillation process.

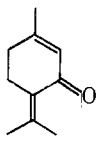
Minor Constituents of *A. filifolia.* Because of the unique carbocyclic system represented by isophorone and lactones **8** and **9**, we were interested in looking for possible biosynthetic precursors in the plant. We carried out an analysis of the minor constituents of the steam distillate and identified borneol, (−)-verbenone, piperitenone, and ketoisophorone (**13**). Of these, compound **13** is the only one with the 1,1,3-trimethylcyclohexane structure.[10] It should also be noted that (−)-verbenone occurs in the plant, whereas (+)-verbenone is the synthetic precursor to lactones **8** and **9** as described above.



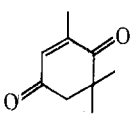
borneol



(−)-verbenone

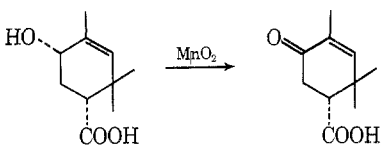


piperitenone



13

The acidic fraction of the chloroform extract was also examined for possible carboxylic acid precursors. The extract yielded a keto acid, (+)-**14**, whose structure was established by MnO_2 oxidation of compound **6**. Compound **14** has not been reported previously as a natural product.

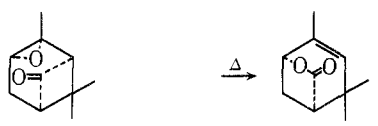


(+)-**6** (+)-**14**

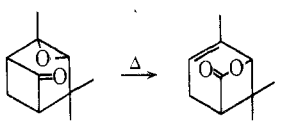
Also found in this fraction was the flavone acacetin.[11] From the neutral chloroform extract the sesquiterpene lactone colartin[12] was isolated.

Discussion

Rearrangements. In the course of the preparation of lactones **8** and **9**, we noted a remarkable thermal rearrangement.

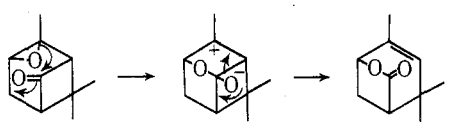


(−)-chrysanthenone epoxide (−)-**8**

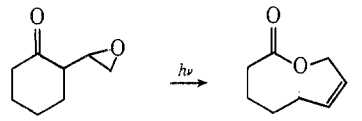


12 **9**

Such a $[_{\sigma}2_s + {}_{\sigma}2_s]$ cycloaddition-type reaction is symmetry forbidden[13] if concerted (*i.e.*, ground state). Thus, the rearrangement probably occurs in a stepwise fashion; the following example is just one of many possible mechanisms.

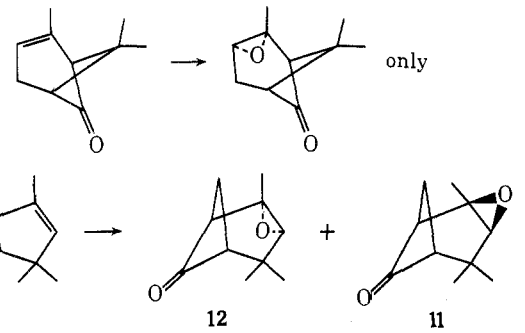


The driving force probably comes from the rupture of the strained cyclobutanone ring system. The reaction may have synthetic applications to other β-epoxy ketone systems. A closed related reaction has recently been carried out by Carlson,[14] the photocatalyzed conversion of **15** to **16**.



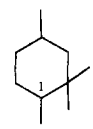
15 **16**

The influence of steric factors was pronounced in the epoxidation of chrysanthenone. In this reaction, only the cis (with respect to the ketone function) epoxide was obtained. On the other hand, epoxidation of compound **10** led to a mixture of cis and trans epoxy ketones.



10 **12** **11**

Biosynthetic Implications. The occurrence of large amounts of isophorone in the plant tissue is unusual, although it has been reported recently[15-17] in small amounts in other species. The carbon skeleton **17** is found



17

in five of the monoterpenes of *A. filifolia*, namely **8**, **9**, **13**, **14**, and isophorone. Altogether they constitute 40% of the monoterpene fraction and represent an important biosynthetic pathway. Presumably, they arise from a common precursor, but one not normally associated with terpene biosynthesis.

The head-to-middle coupling of two isoprenyl pyrophosphate units can account for all of the "irregular" constituents of *A. filifolia*. This mechanism has been proposed[18] for the biosynthesis of lavandulol and related monoterpenes. The stereochemistry at C-1 of **17** in the *A. filifolia* monoterpenes is consistent with that reported by Epstein[18,19] for open-chain analogs of lavandulol in other *Artemisia* species.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Ultraviolet spectra were measured on a Cary recording spectrophotometer, Model 14. Infrared (ir) spectra were recorded on Perkin-Elmer Models 137 and 337. Nmr spectra were measured on Varian Associates A-60A, T-60, and HA-100 instruments and peak positions are given in δ values, using tetramethylsilane as an internal standard. Mass spectra were recorded on a Hitachi Perkin-Elmer Model RMU-6E mass spectrometer. Optical rotations were measured on a Cary Model 60 recording spectropolarimeter by scans from 5800 to 6000 Å, selecting the value of 5893 Å as the sodium D line. Gas chromatography (glc) was carried out on a Varian Aerograph Model 90-P, using a Varian Aerograph Model 20 strip chart recorder. The columns used for glc were 15% SE-30 on Chromosorb P, 20 ft × 0.375 in. aluminum tubing (hereafter referred to as column I); 20% Carbowax 20M on Chromosorb W (acid-washed), 5 ft × 0.25 in. copper tubing (hereafter referred to as column II); and 18% SE-30 on Chromosorb P, 6 ft × 0.25 in. copper tubing (hereafter referred to as column III).

Steam Distillation of the Plant Material. Whole, wet plant material gathered 2 miles southeast of Willcox, Ariz., on Oct 31, 1969, and Aug 6, 1970, was steam distilled as follows. A 2- to 8-kg portion of wet plant was placed in a 10-gallon milk can and the can was half filled with water. A special top with a vertical exit tube was then clamped on the milk can. To this vertical metal tube were then attached three condensers in series. The drum was heated with a Meeker burner until about 4 l. of distillate (cooled in ice) had been collected. This process takes about 4 hr. A visible yellow layer of oil was present on top of the water in the distillate. The steam distillate was extracted with ether and dried over anhydrous sodium or magnesium sulfate, and the solvent was carefully removed *in vacuo* to give the steam-volatile oil as the residue. In some experiments chloroform was used in place of ether as the solvent for the extractions. A total of seven such steam distillations was carried out. The average yield based on wet plant material was 0.8%.

Fractional Distillation of the Steam Distillate. The steam distillate (steam-volatile oil) of *Artemisia filifolia* was distilled *in vacuo* through a 600-mm vacuum-jacketed Vigreux column. The pertinent data are included in Table I.

α-Fencholenic Acid (α,α,3-Trimethylcyclopent-2-ene-1-acetic Acid. α-Fencholenic acid was prepared from (±)-fenchone.[20,21] Much longer reaction times than reported were required in our experiments, especially in the conversion of α-fencholenic acid amide to the acid. α-Fencholenic acid was also prepared from (±)-filifolone.[22] The infrared [(neat) 3100 (very broad) and 1705 cm^{-1}], nmr [(CCl_4) δ 11.69 (br s, -COOH), 5.20 (br s, H-2), 1.6-3.0 (complex, H-1, -4, -4′, -5, -5′), 1.73 (br s, C-3 methyl), and 1.08 (s, α-methyls)], and mass [*m/e* 168 (M^+), 107, and 81 (base, $-(\cdot C_3H_7 + CO_2)$)] spectra of our material were consistent with those reported[22] for α-fencholenic acid.

Anal. Calcd for $C_{10}H_{16}O_2$: C, 71.39; H, 9.59. Found: C, 71.08; H, 9.80.

Lactone 2 (2-Hydroxy-α,α,3-trimethylcyclopent-3-ene-1-acetic Acid γ-Lactone). α-Fencholenic acid was converted to its iodolactone with iodine and sodium bicarbonate.[22]

The iodolactone (1.8 g, 0.0060 mol) of α-fencholenic acid was dissolved in toluene (15 ml). To this solution was added 1,5-diazabicyclo[4.3.0]non-5-ene (DBN, 1.5 g, 0.012 mol) and the reaction mixture was stirred under reflux for 50 min. The mixture was cooled, diluted with water (100 ml), and extracted with ether (2 × 100 ml). The ether extracts were combined and washed with 5% hydrochloric acid (2 × 100 ml), water (100 ml), saturated sodium thiosulfate (4 × 100 ml) until colorless, and then water (100 ml). The ether solution was dried over anhydrous magnesium sulfate and evaporated to yield 1.2 g of a yellow oil. Nmr analysis revealed this oil to be *ca.* 80% lactone **2**; it was subjected to preparative glc (column III, column temperature 170°). The major peak was collected (there appeared to be some thermal decomposition of **2**) resulting in pure lactone **2** as a colorless oil, mp ~10° (0.70 g, 0.0042 mol, 70%). The nmr [(CCl_4) 5.60 (br s, H-4), 5.04 (br d, *J* = 4 Hz, H-2), 2.77 (dd, *J* = 4, 7 Hz, H-1), 2.38 (m, H-5, -5′), 1.82 (d, *J* = 1 Hz, C-3 methyl), and 1.24 and 1.12 (both s, α-methyls)], ir [(neat) 1760 cm^{-1}] and mass [*m/e* 166 (M^+), 122 ($-CO_2$), 107 (base, $-(CO_2 + \cdot CH_3)$), and 91] spectra were in accord with structure **2**.

Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.11; H, 8.63.

Hydrogenolysis of *A. filifolia* Lactones 8 and 9. Carboxylic Acids 3 and 4. Several similar experiments were performed; therefore, only one shall be described in detail here. The products from all such experiments differed only in the relative ratios of **3**:**4**. That is, lactone **9** underwent hydrogenolysis more readily than lactone **8**. Therefore, shorter reaction times gave a **3** + **4** mixture richer in acid **4** than did longer reaction times.

The *A. filifolia* lactone mixture (**8** + **9**) purified by fractional distillation (1.21 g, 0.00730 mol) was dissolved in absolute ethanol (10 ml). To this was added 10% palladium on carbon (0.050 g) and the resulting suspension was hydrogenated at atmospheric pressure and room temperature for 17 hr. The amount of hydrogen absorbed (215 ml) calculates for slightly more than that required (194 ml) for 1 mol of H_2/mol **8** + **9**. The ethanolic mixture was then filtered through Celite, diluted with 5% aqueous sodium hydroxide (100 ml), and extracted with ether (2 × 100 ml). Sodium bicarbonate solution was found to be an ineffective solvent for these β,β-disubstituted (sterically hindered) carboxylic acids (**3** and **4**). Glc analysis of the ether extract revealed cineole, isophorone, camphor, dihydrofilifolone, and unreacted lactones **8** and **9**.

A work-up of the sodium hydroxide extract yielded 0.987 g of acidic product as a yellow oil. The nmr spectrum of this oil showed it to consist of a 3:1 mixture of **3**:**4**. The yield of the **3** + **4** mixture was calculated to be 0.00528 mol (72.2%).

Table I
Fractional Distillation of the *A. filifolia* Steam Distillate

Fraction	Bp, °C	Wt, g	Composition[a]
1	30–46	17.0	Ci:C:F:I (11:1:1:1)
2	48–62	44.3	C:F:I (8:1:1)
3	64–71	6.3	C:I:L:O (20:20:1:7)
4	72–81	9.1	I:L:O (3:3:4)
5	84–90	12.2	L:O (3:2)
6	91–109	9.0	>80% L + some IGA[b]
7	110–118	20.5	>90% IGA[b]

[a] Determined by glc and nmr; Ci = 1,8-cineole, C = camphor, F = filifolone, I = isophorone, L = lactones **8** + **9**, O = others. [b] IGA = isogeranic acid, a thermal decomposition product of filifolone.

Hydrogenation of the Carboxylic Acid Mixture 3 + 4 from *A. filifolia*. Carboxylic Acid 5 (2,2,4-Trimethylcyclohexane-1-carboxylic Acid). The mixture of carboxylic acids of formula $C_{10}H_{16}O_2$, obtained by hydrogenolysis of the *A. filifolia* lactones (above) (0.30 g, 0.0018 mol), was dissolved in 95% ethanol (10 ml). To this was added platinum oxide (0.050 g) and the resulting suspension was subjected to hydrogenation at atmospheric pressure and room temperature for 48 hr. After removal of the catalyst and evaporation of the solvent there remained 0.29 g of a pale yellow oil which could not be made to crystallize. The crude hydrogenation product was purified by its partial conversion to the anilide. The sodium hydroxide extract of the anilide reaction mixture was acidified and extracted with ether, yielding 0.055 g (0.00032 mol, 63%) of crystalline **5**, mp 59–69°. Recrystallization from acetic acid–water gave pure **5** as colorless, tiny flakes, mp 83–84° (lit.[23] mp 81–82°). The acid prepared in this way had nmr [(CCl_4) 11.66 (br s, -COOH), 1.3–2.2 (complex, H-1, -3, -3′, -4, -5, -5′, -6, -6′), 1.03 and 0.98 (both s, C-2 methyls), and 0.89 (d, *J* = 6.5 Hz, C-4 methyl)], ir [($CHCl_3$ 2960 (very broad) and 1710 cm^{-1}] and mass [*m/e* 170 (M^+) and 83 (base)] spectra in accord with structure **5**. In addition, **5** was independently prepared (described later) and shown to be identical with the material described here by identical spectra (ir, nmr) and an undepressed (82–84°) mixture melting point.

Carboxylic Acid 3 (2,2,4-Trimethylcyclohex-3-ene-1-carboxylic Acid). Carboxylic acid **3** was prepared by the thermal Diels–Alder reaction between 2,3-dimethyl-1,3-pentadiene and acrylic acid[7,8,24] and also by the hydrolysis of chrysanthenone.[7] In these reactions, or in their work-up, we observed some isomerization of the product (**3**) to **4**, as noted by Erman.[7] Hence, the observed melting points of our specimens of **3** were about 10° lower than the reported[7] 84–85°. The nmr spectrum, however, of the acid prepared here was identical with that reported[7] for **3**, excepting peaks due to the impurity **4**. The ir [($CHCl_3$) 3000 (very broad) and 1710 cm^{-1}] and mass [*m/e* 168 (M^+), 153 ($-\cdot CH_3$) 123, 107 (base) 96, 81, and 67] spectra were in accord with structure **3**.

Anal. Calcd for $C_{10}H_{16}O_2$: C, 71.39; H, 9.59. Found: C, 70.98; H, 9.47.

Hydrogenation of Synthetic 3. Carboxylic Acid 5. The hydrogenation and subsequent work-up of synthetic **3** were carried out in the same way as the hydrogenation of acids **3** and **4** obtained by hydrogenolysis of *A. filifolia* lactones **8** and **9**. Synthetic **5** had mp 83–84° (lit.[23] mp 81–82°).

Anal. Calcd for $C_{10}H_{18}O_2$: C, 70.55; H, 10.66. Found: C, 70.47; H, 10.59.

Hydrolysis of the *A. filifolia* Lactones 8 and 9. Isolation of Hydroxy Acids 6 and 7 [1(*R*),5(*S*)-(+)-5-Hydroxy-2,2,4-trimethylcyclohex-3-ene-1-carboxylic Acid and 1(*R*),3(*S*)-(−)-3-Hydroxy-2,2,4-trimethylcyclohex-4-ene-1-carboxylic Acid)]. Fraction 6 from the second distillation (see Table I) of the *A. filifolia* steam distillate (9.0 g, 80% **8** and **9**) was dissolved in ether (100 ml) and the ethereal solution was washed with 5% aqueous sodium hydroxide (2 × 100 ml) to remove isogeranic acid. After washing with water (100 ml), drying over anhydrous magnesium sulfate, and evaporation of the ether *in vacuo*, there remained 7.5 g of the mixture of lactones **8** and **9**. Nmr integration revealed this mixture to consist of 85% **8** and 15% **9**. The latter mixture was then placed with potassium hydroxide (4.5 g) in water (150 ml) and refluxed for 140 min. The reaction mixture was cooled, diluted with water (200 ml), and extracted with ether (2 × 100 ml). The ether extracts were combined, dried over anhydrous

magnesium sulfate, and concentrated *in vacuo* to yield 2.9 g of unreacted lactones as a yellow oil. The still-basic aqueous phase was then acidified with hydrochloric acid and extracted with ether (3 × 100 ml). The ether extracts were combined and dried over anhydrous magnesium sulfate and the solvent was removed *in vacuo*. This resulted in 4.5 g of a yellow oil which crystallized on standing. The latter was found by nmr to consist of a 10:1 mixture of **6:7**.

Fractional Crystallization of 6 and 7. The crude crystalline mixture of **6** and **7** (4.5 g) from above was recrystallized from carbon tetrachloride. The first crop of crystals (2.3 g) collected was pure **6**, mp 134–136°. Recrystallization from cyclohexane gave colorless plates, mp 140–141° (lit.[9] mp 140°). This sample of (+)-**6** [1(*R*),5(*S*)-(+)-5-hydroxy-2,2,4-trimethylcyclohex-3-ene-1-carboxylic acid] from *A. filifolia* was optically active, $[\alpha]^{24}D$ +33.8° (*c* 0.5, $CHCl_3$). The infrared [(KBr) 3350, 3000 (very broad), and 1685 cm^{-1}], nmr [($CDCl_3$ + 3 drops DMSO − d_6) δ 5.07 (br s, H-3), 4.00 (br t, J = 7 Hz, H-5), 2.43 (dd, J = 3, 12 Hz, H-1), 1.9–2.2 (m, H-6, -6′), 1.67 (s, C-4 methyl), and 1.11 and 0.97 (both s, C-2 methyls)], and mass [*m/e* 184 (M^+), 166 (− H_2O), 122, and 107 (base, − (H_2O + CO_2 + ·CH_3))] spectra were consistent with structure **6**.

Anal. Calcd for $C_{10}H_{16}O_3$: C, 65.19; H. 8.75. Found: C, 65.20; H, 8.68.

The mother liquor from which pure **6** was crystallized yielded a second crop of crystals (0.8 g). This was shown by nmr to consist of a 3:1 mixture of **7:6**. This material was recrystallized three times from carbon tetrachloride and once from benzene, giving pure (>98%) (−)-**7** (0.16 g) as colorless prisms, mp 134–136°. The hydroxy acid (−)-**7** [1(*R*),3(*S*)-(−)-3-hydroxy-2,2,4-trimethylcyclohex-4-ene-1-carboxylic acid) prepared in this way was optically active, $[\alpha]^{24}D$ −58.0° (*c* 0.9, $CHCl_3$). The nmr [(CCl_4 + 3 drops DMSO-d_6) δ 5.30 (br s, H-5), 3.47 (br s, H-3), 2.24 (m, H-1, -6, -6′), 1.70 (s, C-4 methyl), and 1.00 and 0.93 (both s, C-2 methyls)], ir [(KBr) 3230, 2970 (very broad), and 1680 cm^{-1}] and mass [*m/e* 184 (M^+), 166 (− H_2O), 123, 107 (− (H_2O + CO_2 + ·CH_3))] and 84 (base)] spectra were consistent with structure **7**.

Anal. Calcd for $C_{10}H_{16}O_3$: C, 65.19; H, 8.75. Found: C, 64,95; H, 8.63.

Regeneration of Lactone (−)-8 [1(*R*),5(*S*)-(−)-5-Hydroxy-2,2,4-trimethylcyclohex-3-ene-1-carboxylic Acid γ-Lactone] from the Hydroxy Acid (+)-6. The pure carboxylic acid **6** (1.84 g, 0.0100 mol) from *A. filifolia*, mp 140–141°, $[\alpha]^{24}D$ +33.8°, was heated (neat) in a glass tube in an oil bath at a temperature of 160–175° for 60 min. After this time, the reaction mixture was washed out of the tube with ether (50 ml). The ethereal solution was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to provide 1.64 g (0.00988 mol, 98.8%) of the lactone **8** as a pale yellow oil. This material was homogeneous by glc and its nmr spectrum showed no extraneous peaks. Lactone **8** prepared in this way was optically active, $[\alpha]^{24}D$ −33.2° (*c* 0.4, $CHCl_3$). The nmr [(CCl_4) δ 5.10 (br s, H-3), 4.40 (m, H-5), 2.3 (m, H-1, -6, -6′), 1.80 (d, J = 1 Hz, C-4 methyl), and 1.08 (s, C-2 methyls)], ir [(neat) 1770 and 1670 cm^{-1}] and mass [*m/e* 166 (M^+), 107 (base, − (CO_2 + ·CH_3)), 69, and 41] spectra were consistent with structure **8**.

Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.12; H, 8.41.

Regeneration of Lactone (+)-9 [1(*R*),3(*S*)-(+)-3-Hydroxy-2,2,4-trimethylcyclohex-4-ene-1-carboxylic Acid γ-Lactone] from the Hydroxy Acid (−)-7. This experiment was performed exactly as was the preceding experiment. The carboxylic acid **7** (0.100 g, 0.00543 mol) from *A. filifolia*, mp 134–136°, $[\alpha]^{24}D$ −58°, when heated at 160–175° for 1 hr provided, after work-up, 0.0901 g (0.00543 mol, 100%) of lactone **9** as a pale yellow oil. This material was homogeneous by glc and its nmr spectrum showed no extraneous peaks. Lactone **9** prepared in this way was optically active, $[\alpha]^{24}D$ +43.8° (*c* 1.2, $CHCl_3$). The nmr [(CCl_4) δ 5.43 (m, H-5), 3.87 (br s, H-3), 2.3 (m, H-1, -6, -6′), 1.80 (s, C-4 methyl), and 1.20 and 1.10 (both s, C-2 methyls)], ir [(neat) 1770 and 1650 cm^{-1}] and mass [*m/e* 166 (M^+), 123, 107 (− (CO_2 + ·CH_3)), 95, 83, and 43 (base)] spectra were consistent with structure **9**.

Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.19; H, 8.51.

Photolysis of (+)-Verbenone. Preparation of (+)-Chrysanthenone. Photolysis of (+)-verbenone at a temperature of 50° gave largely racemic products. Therefore, a large fan was used to cool the Hanovia 450 broad spectrum mercury arc lamp employed for the photolysis. This brought the temperature down to 34°. Under these conditions, (+)-verbenone[25] (partially active, $[\alpha]^{24}D$ +93.7°, 37% optically pure) (10.28 g, 0.0685 mol) in cyclohexane (250 ml), in two Vycor vessels, was photolyzed for 21.0 hr. After this time, a glc analysis showed the composition of the reaction mixture to be 40.7% chrysanthenone, 58.2% verbenone, and only traces (<2%) of other products. The cyclohexane was then removed *in vacuo* to provide 11.2 g of a yellow oil. The latter was distilled under vacuum (7 mm) through a 250-mm vacuum-jacketed Vigreux column. Four fractions were collected (Table II).

Table II

Fraction	Bp, °C	Wt, g	Composition[a]
1	74–76	0.04	88% C, 12% V
2	76.5–77	2.57	87% C, 13% V
3	77.5–79.5	1.30	80% C, 20% V
4	80–82	0.85	60% C, 40% V
Pot residue		4.8	80% V + some IGA, I, P

[a] By glc and nmr analysis. C = chrysanthenone; V = verbenone; I = isopiperitenone; P = piperitenone; IGA = isogeranic acid, a thermal decomposition product of chrysanthenone.

Chrysanthenone, from distillation fraction 2, was optically active, $[\alpha]^{24}D$ +31° (*c* 3.7, $CHCl_3$) (corrected for 13% verbenone). This corresponds to an optical purity of 29%, based upon a specific rotation of +108° for pure chrysanthenone.[26] Racemic chrysanthenone was also prepared from verbnone by photolysis at 50°. This material was purified by distillation and preparative glc and had an ir spectrum identical with the reported[27] for chrysanthenone. The nmr spectrum of the pure racemic chrysanthenone was identical with the spectrum reported[26] and also identical with that recorded for the partially optically active material described above, excepting peaks due to the impurity, verbenone, in the latter.

Oxidation of (+)-Chrysanthenone. Preparation of (−)-Chrysanthenone Epoxide. To (+)-chrysanthenone (from distillation fraction 2, above, containing 13% verbenone) (1.08 g, 0.00720 mol) in chloroform (30 ml) was added 85% *m*-chloroperbenzoic acid (1.46 g, 0.00733 mol, based upon 85% purity) and sodium bicarbonate (0.61 g, 0.0072 mol). The resulting mixture was stirred at room temperature under anhydrous calcium chloride for 2 hr. The mixture was then diluted with water (50 ml) and chloroform (50 ml) and shaken and the organic layer was separated. The chloroform solution was washed with 5% sodium hydroxide (2 × 50 ml) and then water (50 ml), dried over anhydrous magnesium sulfate, and concentrated *in vacuo* to provide 1.18 g (0.00710 mol, 99%) of a yellow oil. This was shown by nmr analysis to be chrysanthenone epoxide, contaminated only with verbenone (13 mol %). This material was optically active, $[\alpha]^{24}D$ −26° (*c* 4.8, $CHCl_3$) (corrected for 13 mol %, 12% by weight, verbenone). If the chrysanthenone epoxide prepared here is 29% optically pure, as was its precursor, (+)-chrysanthenone, then the specific rotation (unreported) of pure (−)-chrysanthenone epoxide is −90°. The nmr spectrum of this sample was identical, excepting peaks due to verbenone, with that of racemic chrysanthenone epoxide prepared from pure racemic chrysanthenone: nmr (CCl_4) δ 3.17 (br s, 1 H) and 2.75 (m, 1 H, protons α to ketone carbonyl), 1.9–2.4 (complex, 3 H, methylene and epoxide methine), 1.37 (s, 3 H, CH_3CO–), 1.30 and 1.10 (both s, 3 H, *gem*-dimethyls).

Thermolysis of (−)-Chrysanthenone Epoxide. (−)-Lactone 8. (−)-Chrysanthenone epoxide, $[\alpha]^{24}D$ −26° (still containing 13 mol % verbenone) (0.1683 g, 0.001013 mol), was heated (neat) in a glass tube in an oil bath at 170–180° for 20 min. An nmr spectrum of the product showed it to be lactone **8**, contaminated only with verbenone (13 mol %, 12% by weight). This material was a pale brown oil (0.1648 g, 0.000993 mol, 98%) and was optically active, $[\alpha]^{24}D$ −8.1° (*c* 1.8, $CHCl_3$) (corrected for 13 mol % verbenone). This calculates for an optical purity of 24%. The nmr spectrum of this material was identical (except for verbenone peaks) with that of pure **8** prepared as described elsewhere.

Hydrolysis of (−)-Chrysanthenone Epoxide. (+)-6. (−)-Chrysanthenone epoxide, $[\alpha]^{24}D$ −26° (0.44 g, 0.00265 mol), was refluxed with potassium hydroxide (2.0 g) in 95% ethanol (20 ml) and water (2 ml) for 2 hr. The mixture was then cooled, diluted with water (75 ml), washed with chloroform (2 × 50 ml), acidified, and reextracted with chloroform (2 × 50 ml). The latter chloroform extracts were combined, washed with water (50 ml), dried over anhydrous magnesium sulfate, and evaporated to give 0.0824 g of a pale yellow oil. This oil later crystallized after trituration with ether. The crystals (0.0532 g, 0.000289 mol, 10.9%) were recrystallized from benzene to colorless platelets, mp 136–

Table III

Retention time, min[a]	Compd	Rel peak area	Yield g	Yield %
0.9	1,8-Cineole	35	1.7	26
2.0	Filifolone	10	0.48	7.4
2.6	Camphor	30	1.5	23
3.2	Isophorone	40	1.9	29
4.0	Borneol + **13** (1:1)	2	0.070	1.1
4.7	(—)-Verbenone	1	0.041	0.63
8.6	Piperitenone	2	0.066	1.0
10.2	**8** and **9**	10	0.50	7.7

[a] Flow rate = 100 ml/min, column = 178°, detector = 220°, injector = 210°.

138°. An nmr spectrum of this material was identical with that of **6** prepared as previously described. This sample of the hydroxy acid **6** was optically active, $[\alpha]^{24}D$ +8.2° (*c* 0.56, $CHCl_3$). This calculates for an optical purity of 24%.

Bromination of the Acid 3. Preparation of Lactone 8. 2,2,4-Trimethylcyclohex-3-ene-1-carboxylic acid (**3**, 2.1 g, 0.012 mol) was dissolved in carbon tetrachloride (40 ml), to which was then added *N*-bromosuccinimide (3.1 g, 0.017 mol) and benzoyl peroxide (0.20 g, 0.00083 mol). The mixture was stirred and refluxed under nitrogen for 22.5 hr. Work-up in the usual way afforded no acidic products (except *m*-chlorobenzoic acid). The neutral fraction, after evaporation of the solvent, gave 1.5 g of a yellow oil. An nmr examination of this oil revealed 27% lactone **8**, along with at least two unidentified products. This oil was combined with the oils obtained in the same way from two other brominations of **3** to give a total of 4.0 g of crude lactone **8**. Short-path distillation at reduced (0.3 Torr) pressure gave 1.1 g (0.0066 mol, 20%) of pure **8**, as a colorless oil, bp 79–83°. The nmr and ir spectra of **8** prepared in this way were identical with those of **8** from *A. filifolia*.

Photolysis of Chrysanthenone. Preparation of the Ketone 10 (2,4,4-Trimethylbicyclo[3.1.1]hept-2-en-6-one). Chrysanthenone, obtained by photolysis of verbenone, was photolyzed in substantially the same way, but at 50°. The products were the same as those reported by Erman.[26] The products were collected by preparative glc on column I or column II. The ketone **10** obtained in this way (average yield 20%) had nmr [(CCl_4) δ 5.00 (br s, H-3), 2.8 (m, H-1, -5), 1.9–2.8 (complex, H-7, -7′), 1.76 (d, *J* = 1.5 Hz, C-2 methyl), and 1.09 (s, C-4 methyls)], ir [(neat) 1780 and 1650 cm^{-1}] and mass [*m/e* 150 (M^+), 122 (− CO), 108, 107 (base), 93, 91, and 80] spectra consistent with those reported.[26]

Anal. Calcd for $C_{10}H_{14}O$: C, 79.96; H, 9.39. Found: C, 80.10; H, 9.21.

Oxidation of 10. Preparation of Epoxide 12. To the ketone **10** (0.157 g, 0.00105 mol) in dichloromethane (3 ml) was added *m*-chloroperbenzoic acid (0.21 g, 0.00122 mol) in dichloromethane (15 ml). The reaction mixture was then stirred under anhydrous calcium chloride at room temperature for 20 hr. After work-up there was obtained 0.112 g (0.000675 mol, 64.3%) of crude epoxide product as a pale yellow oil. Nmr analysis of this oil revealed the presence of the two stereoisomeric epoxides **11** and **12**, by glc analysis in the ratio 1:3, respectively. Preparative glc of the **11** and **12** mixture on column I resulted in 0.0420 g (0.000253 mol, 25.1%) of pure **12**. The nmr [(CCl_4) δ 3.10 (m, H-1), 2.52 (s, H-3), 2.5 (m, H-5), 1.8 (m, H-7, -7′), 1.43 (s, C-2 methyl), and 1.17 and 1.07 (both s, C-4 methyls)], ir [(neat) 1780 cm^{-1}] and mass [*m/e* 166 (M^+), 123, and 107 (base)] spectra of this material were in accord with structure **12**.

Epoxide **12**, under the conditions of the mass spectroscopy (inlet temperature 200°), isomerizes largely to lactone **9**.

Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 71.99; H, 8.57.

Pure epoxide **11** was obtained in amounts too small for adequate structural data to be gathered.

Thermal Rearrangement of Epoxide 12. Preparation of Lactone 9. Pure epoxide **12** (0.0420 g, 0.000253 mol) was heated (neat) in an nmr tube in an oil bath at 160–175° for 12 min. The tube was cooled and weighed (0.0419 g, 0.000252 mol, 99.7%) and the nmr spectrum was recorded. The latter was found to be representative of pure **9**, with no extraneous peaks. The infrared spectrum of this sample was identical with that of **9** isolated from *A. filifolia*.

Solvolysis of Epoxide 12. Preparation of Hydroxy Acid 7. Epoxide **12** (0.050 g, 0.00030 mol) was hydrolyzed in the same way as was chrysanthenone epoxide. The usual work-up afforded 0.042 g of a brown oil. The latter was chromatographed over silica gel (20 × 350 mm) and elution with ether gave crystalline **7** (0.029 g, 0.00017 mol, 58%), mp 120–126°. Recrystallization from cyclohexane gave pure **7** (0.022 g) as colorless cubelets, mp 134–136°. That this was identical with the **7** from *A. filifolia* was shown by an undepressed (133–136°) mixture melting point and identical ir and nmr spectra.

Preparative Glc of the *A. filifolia* Steam Distillate. Isolation of (−)-Verbenone, Borneol, Piperitenone, and the Enedione 13. The total steam distillate of *Artemisia filifolia* (6.5 g) was subjected to preparative glc on column II as summarized in Table III.

Identities of these compounds were established by spectral comparisons with authentic specimens and confirmed by mixture melting points of derivatives.

3,5,5-Trimethylcyclohex-2-ene-1,4-dione (13). This compound was prepared from isophorone by the method of Isler.[10] In the last step of the synthesis, activated manganese dioxide was used in place of chromic acid.

Anal. Calcd for $C_9H_{12}O_2$: C, 71.03; H, 7.95. Found: C, 71.27; H, 8.12.

Isolation of Acacetin and Keto Acid (+)-14. *Artemisia filifolia* was extracted with chloroform by percolation and the extract was separated into neutral and acidic fractions by sodium hydroxide extraction thereof. Chromatography of the acidic fraction over silicic acid gave 2% acacetin and 0.4% keto acid (+)-**14** (yields based upon amount of acidic fraction). The latter was recrystallized from benzene to colorless cubelets, mp 106–108°, $[\alpha]^{24}D$ +128° (*c* 1.0, $CHCl_3$). Identity of each of these two compounds was established by taking a mixture melting point with a synthetic specimen (see below).

Acacetin. Synthetic acacetin was prepared from anisic anhydride and phloracetophenone by the method of Robinson and Venkataraman,[11] mp 260–262° (lit.[11] mp 262°).

Anal. Calcd for $C_{16}H_{12}O_5$: C, 65.60; H, 4.25. Found: C, 65.31; H, 4.49.

The diacetate had mp 200–202° (lit.[28] mp 202–203°).

Preparation of the Keto Acid (+)-14 [1(*R*)-(+)-5-Keto-2,2,4-trimethylcyclohex-3-ene-1-carboxylic Acid]. The hydroxy acid **6** ($[\alpha]^{24}D$ +33.8°, from *A. filifolia*) (0.70 g, 0.0038 mol) was dissolved in dichloromethane (100 ml) to which was then added active manganese dioxide (5.0 g, 0.063 mol), prepared according to Ball, Goodwin, and Morton.[29] The resulting suspension was stirred under anhydrous calcium chloride for 24 hr, after which time the reaction mixture was filtered through Celite and the filtrate was evaporated to provide **14** (0.55 g, 0.0030 g, 79%) as a yellow oil, which soon crystallized, mp 101–104°. Recrystallization from benzene afforded pure **14** (0.42 g, 0.0023 mol, 60%) as colorless cubelets, mp 107–109°. The reported melting point[9] for racemic **14** is 107°. The material prepared in this way had nmr [($CDCl_3$) δ 9.6 (br, -COOH), 6.38 (br s, H-3), 2.4–3.0 (complex, H-1, -6, -6′), 1.77 (s, C-4 methyl), and 1.33 and 1.13 (both s, C-2 methyls)], ir [(KBr) 3000 (very broad), 1720, and 1660 cm^{-1}], and mass [*m/e* 182 (M^+) and 137 (base, − (·H + CO_2))] spectra in accord with structure **14**. This material was optically active, $[\alpha]^{24}D$ +128° (*c* 1.05, $CHCl_3$).

Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.92; H, 7.74. Found: C, 65.64; H, 8.06.

Isolation of Colartin. The neutral chloroform extract of *A. filifolia* was chromatographed over silicic acid and elution with 1:1 benzene–chloroform, decolorization, and repeated recrystallizations from hexane gave a 1% yield of pure colartin as colorless, tiny flakes, mp 107–108°, $[\alpha]^{24}D$ +19.4° (*c* 0.88, $CHCl_3$).

Anal. Calcd for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59. Found: C, 71.06; H, 9.67.

A sample of crude colartin[30] (4 mg), mp 81–94°, had an infrared spectrum identical with that of the material from *A. filifolia*. Recrystallization of this authentic specimen from hexane gave colorless, tiny flakes, mp 106–107°. That the material isolated from *A. filifolia* was in fact colartin was confirmed by an undepressed (106–108°) melting point of a mixture of natural and authentic specimens.

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Registry No.—**2**, 50585-68-7; **3**, 13746-43-5; **4**, 50585-69-8; **5**, 50585-70-1; (+)-**6**, 50585-59-6; (−)-**7**, 50585-60-9; (−)-**8**, 50585-61-0; (+)-**9**, 50585-62-1; **10**, 50585-71-2; **11**, 50585-63-2; **12**, 50763-18-3; **13**, 1125-21-9; (+)-**14**, 50585-64-3; acacetin, 480-44-4; colartin,

24493-40-1; 1,8-cineole, 470-82-6; (−)-camphor, 464-48-2; filifolone, 4613-37-0; isophorone, 78-59-1; α-fincholenic acid, 32082-53-4; α-fincholenic acid iodolacetone, 4627-35-4; (+)-chrysanthenone, 38301-80-3; (−)-chrysanthenone epoxide, 50763-19-4; (+)-verbenone, 18309-32-5.

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Mechanism of Cystine Racemization in Strong Acid

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Cystine (1) is the only naturally occurring amino acid which is racemized to a significant extent under the conditions commonly used for hydrolysis of proteins. A study of the mechanism of the racemization of cystine in refluxing 6 *N* HCl is reported. Various analog studies show that the racemization mechanism involves the formation of an acid enol which is stabilized by the inductive effect of a partially or fully charged β-heteroatom. In general, the requisite charge density may be induced through resonance as in the case of *S*-(2,4-dinitrophenyl)-L-cysteine or through protonation as in the case of 2,3-diaminopropionic acid, both of which racemize in acid at rates comparable to that of cystine. However, in the case of cystine, enolization does not occur in the intact molecule as a result of disulfide protonation. Rather, the intermediates in a concurrent, acid-catalyzed, disulfide interchange reaction are implicated as the species which undergo racemization.

All of the α-amino acids undergo some racemization during acid hydrolysis of protein, and the degree to which the various amino acids racemize has been quantitated by tritium incorporation experiments.[1] In the case of every naturally occurring amino acid but one, cystine (1), the rate of racemization is vanishingly slow under normal hydrolysis conditions. Cystine, on the other hand, is almost completely racemized after 120-hr exposure to refluxing 6 *N* HCl. When cystine, the first amino acid discovered, was initially isolated from kidney stones in 1810,[2] it was obtained in a relatively high state of optical purity, as the isolation did not involve extended heating in strong acid. It was not until 89 years after its discovery that cystine was isolated from horn hydrolysate[3] and shown to be the source of much of the sulfur known to be present in protein. In this very early work, the rotation of the cystine isolated was found to be dependent upon the duration of hydrolysis. In 1902,[4] the correct structure of cystine was elucidated and it was shown to be racemized completely by heating in hydrochloric acid. The existence of D, L, and meso forms was proposed in the inactive material, and all three forms were isolated in 1933.[5]

Somewhat surprisingly, in none of the above work or in other studies of this unique racemization[6] is there comment or speculation about the mechanism of the reaction. In the course of preparing a sample of DL-cystine for a separate study, our curiosity was aroused by the anomalous rate at which L-cystine racemized in acid, and we have examined this reaction with the objective of understanding its mechanism.

The decrease in rotation of a solution of cystine in refluxing 6 *N* HCl is first order in amino acid and has a half-life of about 20 hr. Unfortunately, dependence of the rate on acid concentration could not be fully investigated because of the rapid decomposition of cystine which occurs in more dilute, refluxing acid. Specifically, it was found that in refluxing 1 or 3 *N* HCl rather rapid decomposition occurred to give cysteine, alanine, glycine, and other products, production of which occurred rapidly enough to render racemization rate measurements meaningless. The extent of decomposition which occurred during racemization experiments was monitored by amino acid analysis and it was found that in 5 *N* or stronger HCl, decomposition occurred only very slowly.

The effect of the conjugate base of the mineral acid on racemization rate was investigated by comparing the results of experiments conducted in HCl, HBr, and H_2SO_4 at 110° (sealed tube) and constant H_0. Though these acids have widely variant anion nucleophilicities, the rate of racemization was essentially constant for all three. This ob-