The Sesquiterpenoid Lactones of *Helenium Bigelovii* Gray

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Four sesquiterpenoid lactones have been isolated from *Helenium Bigelovii* Gray. Three of these have been characterized as the known compound tenulin, isotenulin, and desacetylisotenulin, of which the latter two may be artifacts of the isolation procedure. The fourth, bigelovin, is new and has been assigned the structure VII. Bigelovin possesses the ambrosin skeleton, now recognized as of frequent occurrence among the azulenogenic lactones of Compositae.

The genus *Helenium* (Compositae) has proved to be a rich source of sesquiterpenoid lactones of the perhydroazulene group. Among the known compounds of this type that have been isolated from the genus are helenalin,[1,2,9] tenulin and isotenulin,[3-9] pinnatifidin,[10] neohelenalin,[11] the brevilins A, B, and C,[10] and the mexicanins A, B, C, and D.[12] In addition to these C_{15} compounds (and their acetates), there have been found the norsesquiterpenoid lactone, mexicanin E,[13] from *H. mexicanum*, and two compounds from *H. flexuosum* which may be monocarboxyclic lactones.[11] The isolation of helenalin, along with balduilin, from *Balduina uniflora* is the only reported case of the occurrence of helenalin outside of the genus *Helenium*.[14]

Helenium Bigelovii Gray is a perennial montane species in California, the leaves of which are characteristically intensely bitter. Extraction of the dried, whole aerial parts of the plant, collected at about the time of flowering, with chloroform yielded a bitter, oily mixture of the lactonic constituents, partially crystalline in the crude state. Tenulin was separated as its benzene solvate and the residual mixture was chromatographed on neutral alumina. Bigelovin, isotenulin, and desacetylisotenulin were separated by serial elution with benzene, mixtures of benzene and ether, and finally ether.

Tenulin (I),[15] the major constituent, was isolated in amounts of 0.18–1.13% of the dry weight of the plant. It showed the reported aberrant melting point behavior but was identified by its composition ($C_{17}H_{22}O_5$), optical rotation ($[\alpha]^{25}D$ −22.7°), spectral properties [ultraviolet (max.) 225 mμ, ε 7000; infrared 3520 (OH), 1765 (γ-lactone), 1700 and 1580 (cyclopentenone) cm.$^{-1}$], and its ready conversion into isotenulin, the properties of which were in agreement with those reported.[4]

Isotenulin (II) was obtained from benzene–ether eluates of the alumina columns and was identical with material prepared by isomerization of tenulin with Los Angeles tap water.

It is probable that isotenulin is an artifact of the separation on alumina; the conditions under which tenulin is isomerized are very mild,[4] and neutral alumina might serve to catalyze this conversion. The fact that tenulin was never found in column eluates, even though it is very unlikely that it was removed completely by crystallization from the crude lactone mixture, supports this view.

Several derivatives of isotenulin [dihydroisotenulin (III), desacetyldihydroisotenulin (IV), dehydrodesacetyldihydroisotenulin (V), and desacetylisotenulin (VI)] were prepared in the course of attempts to find a way to correlate bigelovin with tenulin. The correspondence of the properties of these compounds with those described in the literature left no doubt as to their identity.

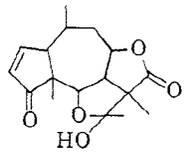
Desacetylisotenulin (VI) was present in ether eluates of the first alumina columns and was separated from traces of isotenulin by repeated chromatography on neutral alumina (activity IV). It was identified by comparison with the compound prepared from isotenulin, and by its conversion into desacetyldihydroisotenulin. There is no corollary evidence that desacetylisotenulin actually occurs in the plant; there is a possibility that it is formed by deacetylation of isotenulin during the chromatographic manipulations.

Bigelovin (VII) was obtained in amounts that varied, with different batches of plant material, from 0.01 to 0.2% of dry weight of plant. Its melting point (190–191°) and composition (C_{17}-

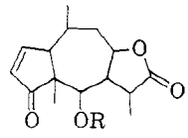
(1) R. Adams and W. Herz, *J. Am. Chem. Soc.*, **71**, (a) 2546, (b) 2551, (c) 2554 (1949).
(2) G. Büchi and D. Rosenthal, *ibid.*, **78**, 3860 (1956).
(3) E. P. Clark, *ibid.*, **62**, 597, 2154 (1940).
(4) D. H. R. Barton and P. DeMayo, *J. Chem. Soc.*, 142 (1956).
(5) H. E. Ungnade and E. C. Hendley, *J. Am. Chem. Soc.*, **70**, 3921 (1948).
(6) H. E. Ungnade, E. C. Hendley, and W. Dunkel, *ibid.*, **72**, 3818 (1950).
(7) B. H. Braun, W. Herz, and K. Rabindran, *ibid.*, **78**, 4423 (1956).
(8) C. Djerassi, J. Osiecki, and W. Herz, *J. Org. Chem.*, **22**, 1361 (1957).
(9) W. Herz and R. B. Mitra, *J. Am. Chem. Soc.*, **80**, 4876 (1958).
(10) W. Herz, R. B. Mitra, K. Rabindran, and W. A. Rohde, *ibid.*, **81**, 1481 (1959).
(11) W. Herz, P. Jayaraman, and H. Watanabe, *ibid.*, **82**, 2276 (1960).
(12) A. Romo de Vivar and J. Romo, *Chem. Ind.* (London), 882 (1959).
(13) A. Romo de Vivar and J. Romo, *J. Am. Chem. Soc.*, **83**, 2326 (1961).
(14) W. Herz, R. B. Mitra, and P. Jayaraman, *ibid.*, **81**, 6061 (1959).

(15) The structure written for tenulin is the revised structure alluded to by W. Herz, M. Miyazaki, and Y. Kishida,[18] and described in detail by W. Herz, W. A. Rohde, K. Rabindran, P. Jayaraman, and N. Viswanathan, *J. Am. Chem. Soc.*, **84**, 3857 (1962). We are grateful to Prof. Herz for a copy of this paper in manuscript, and for the information (also referred to in the latter paper) that helenalin, balduilin, and the mexicanins also possess the ambrosin skeleton. The structures we write for these compounds are those communicated to us by Prof. Herz.

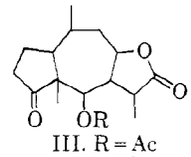
$H_{20}O_5$) distinguished it clearly from the accompanying tenulin derivatives. The ultraviolet [max. 215 mμ (ε 13,000), 321 mμ (ε 45)] and infrared, [1755 (γ-lactone), 1730, 1240 (acetyloxy), 1705 (cyclopentenone), 1660 (C=C) and 1585 (C=C) cm.$^{-1}$] spectra of bigelovin suggested that the lactone ring was α,β-unsaturated (exocyclic methylene). This was later established by other evidence. Bigelovin provided one mole of acetic acid upon either acidic or basis hydrolysis. It is very unstable in alkaline solution, decomposing at once with the development of a deep red coloration; no crystalline product could be recovered after acidification.



I



II. R = Ac
VI. R = H



III. R = Ac
IV. R = H
V. OR = O=

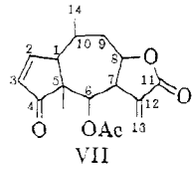
The ultraviolet absorption spectrum of bigelovin resembled those of helanalin, ambrosin and parthenin, all of which, like bigelovin, show absorption maxima between 215 and 220 mμ with ε values of 12,000–16,000. This observation, coupled with the elementary composition, suggested that bigelovin possessed both a cyclopentenone ring and a γ-lactone ring containing an exocyclic methylene group.

Bigelovin readily absorbs two moles of hydrogen to yield tetrahydrobigelovin (VIII), $C_{17}H_{24}O_5$ [no significant ultraviolet absorption; infrared 1765 (lactone), 1740 (cyclopentanone, acetyl), 1230 (acetate) cm.$^{-1}$]. When the hydrogenation was interrupted after the absorption of one mole of hydrogen, dihydrobigelovin (IX), $C_{17}H_{22}O_5$, was obtained. Dihydrobigelovin had λ_{max} 210 mμ (ε 8440) and infrared absorption peaks at 1760, 1742, 1662, 1408, and 1235 cm.$^{-1}$. The movement of the carbonyl band of bigelovin (1705 cm.$^{-1}$) to 1742 cm.$^{-1}$ in dihydrobigelovin and the disappearance of the 1585 cm.$^{-1}$ band showed that, unexpectedly, the cyclopentenone ring had been reduced in dihydrobigelovin, the unsaturated lactone remaining. This behavior is in contrast to that of helenalin and neohelenalin, in which the exocyclic double bond is the first to be reduced.[1c] The spectra of dihydrobigelovin are in accord with structure IX.

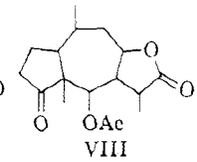
Bigelovin and dihydrobigelovin showed Kuhn–Roth C-methyl values that indicated the presence of three C-methyl groups (one of which is in the acetyl group). Tetrahydrobigelovin showed four. Oxidation of bigelovin and dihydrobigelovin by the Lemieux procedure[16] yielded 0.41 and 0.44 mole of formaldehyde, respectively. Xanthinin,[17] a compound known to contain the same kind of exocyclic methylene group, yielded 0.47 mole of formaldehyde by this procedure. An attempt to prepare a monopyrazoline derivative by the addition of diazomethane to bigelovin was not successful; the crystalline product was difficult to purify, but gave analytical figures that corresponded to the addition of two moles of diazomethane.

The difference between the ultraviolet absorption spectra of bigelovin and dihydrobigelovin showed a maximum at 224 mμ (ε 6400) for the cyclopentenone ring. This is consistent with the structure (VII) proposed.

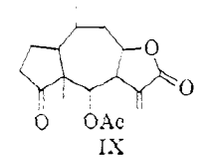
While these studies were in progress, and structure VII had been written for bigelovin on the basis of the n.m.r. evidence described below, Herz[18] reported that the structures that had earlier been assigned to ambrosin (X), damsin (XI), and parthenin (XII) were incorrect, and that these compounds in fact possessed the structures shown:



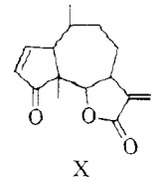
VII



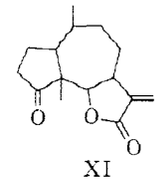
VIII



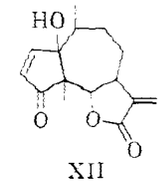
IX



X



XI



XII

During the early stages of the present study when it was apparent that bigelovin and O-acetylhelenalin were isomeric, attempts were made to correlate them by the conversion of bigelovin into a helenalin derivative. Since bigelovin and tenulin occur together, and since tenulin and helenalin have been structurally correlated,[9] it appeared likely that bigelovin differed from O-acetylhelenalin either in the position of the lactone ring closure or in the stereochemistry at 6 or 8. All derivative products of helenalin and tenulin in which asymmetry at C-6 and C-8 has been destroyed are known.

Bigelovin is very unstable under both acidic and basic conditions, and desacetylbigelovin could not be obtained. Tetrahydrobigelovin, too, was unstable to hydrolysis, and its desacetyl derivative could not be obtained by usual procedures. It is noteworthy that aqueous sodium bicarbonate, which readily hydrolyzes isotenulin, did not affect tetrahydrobigelovin. When tetrahydrobigelovin was treated with sodium methoxide in absolute methanol there was obtained in one experiment a small amount of desacetyltetrahydrobigelovin,

(16) R. U. Lemieux and E. von Rudloff, *Can. J. Chem.*, **33**, 1710 (1955).

(17) T. A. Geissman, *J. Org. Chem.*, **27**, 2692 (1962).

(18) W. Herz, M. Miyazaki, and Y. Kishida, *Tetrahedron Letters*, No. **2**, 82 (1961).

$C_{15}H_{22}O_4$, infrared bands at 3520–3540, 1760 and 1725 cm.$^{-1}$.

The unusual behavior of bigelovin on hydrogenation and its greater susceptibility to decomposition by alkali than that reported for helenalin seemed inconsistent with a structure that differed from O-acetylhelanalin only in a stereochemical detail. It was at this time that an examination of the n.m.r. spectrum of bigelovin disclosed that bigelovin and its derivatives possessed the carbon skeletal arrangement shown in VII–XII. In light of the later disclosure[15] that helenalin also possesses this carbon skeleton it is difficult to account for the difference between the behavior of bigelovin and helenalin toward alkali.

The n.m.r. spectrum of bigelovin shows four vinyl protons, two of them present in the exocyclic methylene group of the lactone, and two present in the cyclopentenone ring. The proton at C-3 gives rise to a doublet at 364/375 c.p.s. (coupling with the C-2 proton) which is further split by interaction with the proton at C-1. The C-2 proton also shows a split doublet in the same region (463/472 c.p.s.). Both of these bands have an intensity of one proton each. These doublets are missing from the n.m.r. spectra of dihydro- and tetrahydrobigelovin. Ambrosin shows similar split doublets at 364/373 and 446/454 c.p.s. The exocyclic methylene group of bigelovin appears as a two-proton doublet at 354–375 c.p.s., split by interaction with the C-7 position.

The most revealing feature of the spectra of bigelovin and its derivatives is the appearance of a sharp three-proton singlet at 72 c.p.s., a clear indication that there is present a tertiary methyl group. The secondary methyl group at C-10 appears as the expected doublet at 73/82 c.p.s.

These results are accommodated by the structure VII for bigelovin and cannot be reconciled with the "regular" (guaianolide) arrangement of carbon atoms previously written for tenulin and its congeners.[4] The correspondence between the n.m.r. spectra of bigelovin and many of the compounds in the tenulin–helenalin group[15] supports this assignment.[19]

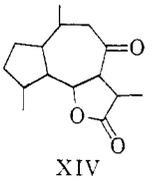
The direction of lactone ring closure shown in VII is so written because of the close correspondence between the n.m.r. spectra of bigelovin and balduilin. The comparison of the signals assigned to the hydrogen atoms on C-6 and C-8 is as follows (c.p.s. relative to tetramethylsilane):

	Balduilin[19]	Bigelovin
C-8—H	271, 274, 280, 283, 286, 289, 292	268, 271, 278, 281, 290, 293
C-6—H	358, 361	334, 342

(19) Prof. Herz (private communication of results submitted for publication) has assigned the same gross structure (VII), to balduilin, and also to a new lactone, linifolin A. Thus, there appears to be a group of similarly constituted compounds which must differ only in stereochemical detail.

The multiplet signals for the C-8 positions are nearly identical for both compounds, while the doublets for C-6 differ by a significant amount, which may indicate that the configuration at C-8 is the same in balduilin and bigelovin but that differences exist at C-5, C-6 or C-7. The bands associated with the proton on C-6, the position of the acetoxyl group, shifted on hydrogenation to tetrahydrobigelovin, and appear in the latter compound at 318, 326 c.p.s.[20] This difference of 16 c.p.s. is exactly matched by that between balduilin and tetrahydrobalduilin.

Further evidence for the structure of bigelovin was obtained from deuterium exchange experiments with tetrahydrobigelovin. The compound of structure VIII would be expected to exchange a maximum of three protons, while the corresponding alternative 3-keto-4-methyl ("regular") structure possesses four exchangeable protons. Herz and Ueda[21] treated dehydrodesacetyldesoxotetrahydromatricarin (XIV) with deuterium chloride in



XIV

deuterium oxide–dioxane and found that 2.47 (61% of three) of the protons were exchanged, the α-hydrogen atom of the lactone ring exchanging only to the extent of about 1%. Tetrahydrobigelovin (which was destroyed by alkali) was equilibrated with a deuterium oxide–deuteriosulfuric acid mixture in dioxane, and showed a maximum incorporation of 1.77 D (59% based on VIII). This compares favorably with the result of the exchange experiment reported for the matricarin derivative and supports the structure that is assigned to bigelovin.

The sensitivity of bigelovin and its reduction products to the action of alkali is now understandable. The β-position of the hydroxyl group at C-6 to the carbonyl group provides for a cleavage of the retroaldol type, leading to a consequent destruction of the compound by further base-induced transformations. The observed instability of ambrosin and damsin to alkali,[22] noted before the revised structures were proposed, is of course explainable on similar grounds.

Since bigelovin, helenalin, and tenulin (see ref. 15) all possess ambrosin-like skeletons, it is puzzling that the order of hydrogenation of the double bonds

(20) The first sample of dihydrogibelovin used for n.m.r. analysis contained traces of the tetrahydro compound as impurity. While the analysis of the resulting complex band pattern was easily made, detailed discussion of regions influenced by this impurity is omitted here, since the spectra of bigelovin and the tetrahydro compound yielded the information necessary for structure analysis.

(21) W. Herz and K. Ueda, *J. Am. Chem. Soc.*, **83**, 1139 (1961).

(22) L. Bernardi and G. Büchi, *Experientia*, **13**, 466 (1957).

in helenalin differs from that in bigelovin, and that the alkali-instability of bigelovin is not matched by tetrahydrohelenalin and tenulin and their derivatives. It is noteworthy that helenalin, but not tetrahydrohelenalin, undergoes decomposition, with the formation of a red color, when treated with alkali.[1a] It is possible that these differences are stereochemical in nature, but with the paucity of present information regarding the stereochemistry of the natural guaianolides, more evidence is needed before satisfactory explanations for these differences can be found.

Experimental

All melting points are corrected, and were determined on a Fisher–Johns melting point apparatus. All infrared spectra are in chloroform solution unless otherwise noted, and were measured on a Perkin–Elmer Model 21 spectrophotometer. Ultraviolet spectra are in 95% ethanol and were determined on a Cary Model 14M spectrophotometer.

Extraction.—Dry, ground aerial parts of *Helenium Bigelovii* Gray plants were soaked in chloroform at room temperature for 24 hr. The chloroform extract was filtered and re-extraction with chloroform was continued until all bitterness was extracted. The combined extracts were evaporated to dryness under reduced pressure; the residual sludge was dissolved in ethanol and three volumes of hot water were added. The mixture was shaken, the tar was allowed to settle, Celite was added, and the supernatant liquid was filtered through a Celite pad. Tar was reextracted several times in the same way with ethanol and water. The combined filtrates were decolorized with charcoal, salt was added, and the solution was exhaustively extracted with chloroform. The dried extract, on distillation to dryness, yielded a yellow, bitter oil (1.2–3.6% dry weight).

Isolation of Tenulin.—The crude yellow oil was dissolved in a minimum amount of hot benzene. On cooling, white crystals were deposited. By evaporation of the filtrate, a second crop of crystals could be obtained. The average total yield was 0.8% of the dry weight of plant material. Repeated recrystallizations from ethyl acetate–petroleum ether produced rhombic crystals with the melting points of various preparations varying from 184–186° up to 195–197°; $[\alpha]_D$ −22.7° (EtOH); λ_{max} 225 mμ (ε 7000), infrared: 3520, 1765, 1700, 1580 cm.$^{-1}$ [lit.,[4] m.p. 192–215°, $[\alpha]_D$ −20 to −24°, λ_{max} 226 mμ (ε 7000), infrared: 3620, 1772, 1708, 1595 cm.$^{-1}$].

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.66; H, 7.19. Found: C, 67.21; H, 7.28.

Chromatography. A. Preparation of Alumina.—Commercial alumina (Harshaw) was neutralized with ethyl acetate, washed with water and methanol, and activated by heating at 150° for 24–28 hr. The activity was found to be I using the Brockmann procedure.[23] Activities III and IV were prepared by adding the appropriate weight of water[24] to activity I alumina. The activities were checked using the Brockmann procedure.

B. Chromatography of Lactone-containing Oils.—The oils remaining after the removal of most of the tenulin by crystallization were chromatographed on alumina columns which were prepared by packing as benzene slurries. The elution order used was benzene, benzene–ether, and ether. Activity IV alumina proved to be the best grade for good separation and recovery of the components of the oils. The best separations were obtained using 2–9 g. of oil per 100 g. of alumina. Benzene eluted bigelovin. Benzene–ether eluted isotenulin, sometimes mixed with bigelovin. Benzene–ether and ether eluted crystalline mixtures in the melting point range 150–160°. When these were rechromatographed on activity IV alumina, benzene–ether eluted a colorless oil, from which isotenulin was crystallized. Ether eluted an oil, from which crystals, m.p. 237–239°, were isolated. This material showed infrared peaks (KBr) at 3340, 1740, 1690, and 1580 cm.$^{-1}$, and proved to be desacetylisotenulin. When activity III alumina was used, the compounds isolated were purer than those from activity IV alumina, but the recovery was poor.

Bigelovin (VII).—Bigelovin was isolated from benzene or benzene–ether eluates of activity III or IV alumina columns. The amount obtained varied with the plant source and with the grade of alumina used; yields varied from 0.01 to 0.2% of the dry weight of plant. Bigelovin crystallizes well from ethyl acetate–petroleum ether, forming white rhombs or rods, and when pure it has no bitter taste. It is quite soluble in chloroform, ethanol, ether and benzene, and insoluble in water and saturated hydrocarbons. It dissolves in bases and concentrated acids with various colorations, and decomposition in all cases. It has m.p. 190–191°; $[\alpha]_D$ +46.1° (EtOH); λ_{max} 215 mμ (ε 13,000), 321 mμ (ε 48), infrared: 1755, 1730, 1705, 1660, 1585, 1240 cm.$^{-1}$.

Anal. Calcd. for $C_{17}H_{20}O_5$: C, 67.11; H, 6.58. Found: C, 67.45, 67.29; H, 6.67, 6.70.

Dihydrobigelovin (IX).—A solution of 0.195 g. of bigelovin in 30 ml. of ethyl acetate was hydrogenated with Adams' catalyst at atmospheric pressure. Hydrogenation was stopped after an uptake of about 1 mole (45 min.). After work-up in the usual manner, fine white needles were obtained from ethyl acetate–petroleum ether, melting at 187–188°, mixed melting point with bigelovin, 185–187°. The yield of pure compound was 0.074 g. The ultraviolet spectrum showed λ 210 mμ (ε 7180), 220 (ε 5590), with an apparent maximum at 210 mμ. The compound had $[\alpha]_D$ +113.4° (EtOH), and infrared absorption at 1760, 1742, 1662, 1408, 1235, 885 cm.$^{-1}$.

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.66; H, 7.19. Found: C, 66.01; H, 7.38.

Tetrahydrobigelovin (VIII).—A solution of 1.0 g. of crude bigelovin in 50 ml. of ethanol was hydrogenated at atmospheric pressure with Adams' catalyst until there was no further uptake of hydrogen (about 1.5 moles). White needles (0.5 g.) were obtained from ethyl acetate–petroleum ether, m.p. 203–205°; recrystallization raised the melting point to 206–207°; $[\alpha]_D$ +181° ($CHCl_3$); infrared: 1765, 1740, 1230 cm.$^{-1}$; no ultraviolet maximum above 210 mμ.

Anal. Calcd. for $C_{17}H_{24}O_5$: C, 66.23; H, 7.79. Found: C, 66.00; H, 7.66.

Attempted Aqueous Hydrolysis of Tetrahydrobigelovin.—A. Tetrahydrobigelovin (0.78 g.) in 75 ml. of water containing 2.2 g. of sodium bicarbonate was refluxed for 2 hr. On acidification with sulfuric acid and cooling, yellowish needles (0.2 g.) were obtained. Purification of this compound showed that it was starting material. The filtrate from the acidified reaction mixture was extracted with ether. A pungent oil was obtained, from which no crystalline material could be obtained.

B. Tetrahydrobigelovin (0.2 g.) was refluxed with 3 ml. of 5% sodium hydroxide for 5 min. The yellow solution was cooled and acidified. A minute amount of crystalline material (m.p. 216°) was obtained. Benzene extraction of the aqueous filtrate provided an oil, from which no crystals were obtained. The infrared spectrum [3700, 3510 (broad), 1775, 1737, 1707 (sh), 1637, 1612 cm.$^{-1}$] indicated that considerable decomposition had occurred.

C. A solution of 0.13 g. of tetrahydrobigelovin in 2 ml. of 25% aqueous *p*-toluenesulfonic acid was heated for 90 min. Steam distillation of the reaction mixture showed that 21% of the acetyl groups had been removed. One milliliter of concentrated sulfuric acid was added to the solution and it was allowed to stand for 2 days, after which 81% of the

(23) H. Brockmann and H. Schodder, *Ber.*, **74**, 73 (1941); P. B. Muller, *Helv. Chim. Acta*, **26**, 1945 (1943).

(24) G. Hesse, I. Daniel, and G. Wohlleben, *Angew. Chem.*, **64**, 103 (1952).

expected acetic acid was detected in the distillate. After distillation the residual solution was cooled, filtered, and extracted with ether. The ether extract was washed with 5% sodium bicarbonate, and water, then dried. The residue from the ether extraction was recrystallized from ethyl acetate–petroleum ether. Starting material (0.05 g.), m.p. 206–207°, was obtained.

D. Tetrahydrobigelovin (0.1 g.) was dissolved in a little concentrated sulfuric acid and the solution warmed on the steam bath for 5 min. The deep yellow solution was poured over ice, neutralized with sodium carbonate to pH 2–3, and extracted with chloroform. A yellow oil was obtained which had infrared bands at 3540, 1765, 1735, 1687, and 1615 cm.$^{-1}$. This oil was chromatographed on 50 g. of activity IV alumina. Benzene eluted an oil with infrared bands at 1750, 1692, and 1610 cm.$^{-1}$. Ether extraction of the alumina yielded an oil with infrared bands at 3570, 1775, 1730, and 1640 cm.$^{-1}$. Neither oil would crystallize; the infrared spectra indicated that dehydration had taken place.

Desacetyltetrahydrobigelovin.—To a solution of 0.1 g. of tetrahydrobigelovin in 10 ml. of dry methanol was added 40 mg. of sodium. The mixture was refluxed 5 hr. under anhydrous conditions and then allowed to stand overnight. The almost colorless solution was diluted with water, immediately neutralized with sulfuric acid to pH 2, and extracted with ether; a light yellow oil was obtained. Crystals melting at 187–189° (impure starting material) were obtained with ethyl acetate–petroleum ether. White crystals (30 mg.), melting at 221–223° after recrystallization from ethyl acetate–petroleum ether, were obtained from the mother liquor. The compound had $[\alpha]_D$ +133° ($CHCl_3$); infrared: 3520–3540, 1760, 1725 cm.$^{-1}$.

Anal. Calcd. for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.54; H, 8.18.

Pyrazoline Derivative from Bigelovin.—Diazomethane, generated from 1.0 g. of nitrosomethylurea, was passed into a solution of 0.2 g. of bigelovin in 50 ml. of ether containing enough dioxane for complete solution. The oil obtained from the reaction mixture had λ_{max} 325, 211, and 225 mμ; it was dissolved in ether containing some 95% ethanol, and petroleum ether was added. On cooling to 0°, yellowish crystals formed; these had m.p. 182° (with bubbling). Recrystallized from ethyl acetate–petroleum ether, the compound melted at 182–183° (bubbles), and showed λ_{max} 213, 234, 236 mμ; infrared: 1770–1765, 1750–1740, 1550, 1235, 885 cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{22}O_5N_2$: C, 62.40; H, 6.36. Calcd. for $C_{19}H_{24}O_6N_4$: C, 58.76; H, 6.19. Found: C, 58.09; H, 6.73.

Basic Hydrolysis of Tetrahydrobigelovin in the Presence of Silver Oxide.—To a solution of 0.1 g. of tetrahydrobigelovin in 5 ml. of 5% sodium hydroxide was added about 50 mg. of silver oxide; the mixture was warmed on the steam bath for 45 min., diluted with water and filtered. The yellow-orange filtrate was acidified with dilute nitric acid and extracted with ether. The ether extract was washed with 5% sodium bicarbonate, then water. The ether yielded a neutral residue with infrared bands at 3700, 1772, and 1730 cm.$^{-1}$; this corresponds to desacetyltetrahydrobigelovin but the residue could not be induced to crystallize. The bicarbonate extract and water washings were reacidified and extracted with ether. The ether extract yielded an acidic material with infrared: 3700, 1780, 1740, 1610 (broad) cm.$^{-1}$; no crystals could be obtained.

C-Methyl Determinations.—C-Methyl numbers were determined according to the method described in Smith and Shriner.[25] The results obtained were as follows: tenulin, 2.8; xanthinin, 2.3 (lit.,[26] 2.45); bigelovin, 2.23; dihydrobigelovin, 2.05, 2.4; tetrahydrobigelovin, 3.19.

Terminal Methylene Determinations.—Exocyclic methylene groups were determined by oxidation to formaldehyde according to the Lemieux method.[16] The formaldehyde was determined spectrophotometrically with chromotropic acid at 570 mμ; erythritol was used to prepare a standard curve. Because of the insolubility of the terpenes in water, 2 ml. of purified dioxane was added at the beginning of the procedure to dissolve the compounds. The same amount of dioxane was added to the solutions used to prepare the standard curve. Neutral oxidation was found to be more satisfactory than alkaline oxidation. The results obtained (μmoles formaldehyde/μmoles compound) are as follows: xanthinin, 0.47; bigelovin, 0.41; dihydrobigelovin, 0.44.

Deuterium Exchange.—Deuteriosulfuric acid was prepared by the method of Schubert and Burkett.[27] The procedure of Herz and Ueda[19] was used for the deuteration. Fifty milligrams of tetrahydrobigelovin was dissolved in 10 ml. of dioxane; 1 ml. of deuterium oxide and 10 drops of deuteriosulfuric acid solution were added. The solution was warmed at 50° under a dry nitrogen atmosphere for 2 days. Solid sodium carbonate was added to neutralize the acid, and the solvent removed *in vacuo*. Ether was added to the residue, and the salt filtered off. The purified deuterated tetrahydrobigelovin, crystallized from ether–petroleum ether, had m.p. 206–207°.

Anal. Calcd. for 2 D: 8.33. Found: 6.22, 7.39 (atom % excess D).

Isotenulin.—A. Isotenulin was prepared by the method of Barton and deMayo[4] in 61% yield. It had m.p. 159–161°; λ_{max} 225 mμ; infrared: 1760, 1745, 1705, 1587, 1230, cm.$^{-1}$ [lit.,[4] 161–162°; $[\alpha]_D$ +4 to +9°; λ_{max} 226 mμ (ε 7000); infrared: 1778, 1748, 1705, 1588, 1238 (Nujol)].

B. Isotenulin was also isolated by chromatography of the crude lactone mixture, appearing in benzene–ether eluates: m.p. 159–160°; infrared: 1745–1755, 1705, 1585, 1240 cm.$^{-1}$; $[\alpha]_D$ +10° ($CHCl_3$). It gave no depression in a mixed melting point with isotenulin prepared from tenulin.

Desacetylisotenulin.—A. The method of Clark[3] was used. Recrystallized from ethyl acetate–petroleum ether, the compound had melting point 245–248°; λ_{max} 226 mμ (ε 9400); $[\alpha]_D$ −15.6° ($CHCl_3$); infrared (KBr): 3440, 1740, 1690, 1580 cm.$^{-1}$.

B. Desacetylisotenulin was isolated from the plant material, appearing in ether eluates of alumina columns. The melting point was 237–239°, and a mixed melting point with the authentic compound was 245–251°. It had λ_{max} 226 mμ; infrared (KBr): 3440, 1745, 1690, 1650 (sh), 1580 cm.$^{-1}$ [lit.,[3,4] m.p. 255°; $[\alpha]_D$ −20°; λ_{max} 225 mμ (ε 8500)].

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16, H, 7.63. Found: C, 68.24, 68.14; H, 7.93, 7.90.

Dihydroisotenulin.—Isotenulin was hydrogenated in ethanol with Adam's catalyst at atmospheric pressure. One mole of hydrogen was taken up (1 hr.). The mixture was worked up in the usual manner. Recrystallized from ethyl acetate–petroleum ether, the product melted at 151–151.5° and had $[\alpha]_D$ +114° ($CHCl_3$); infrared: 1750, 1740, 1245 cm.$^{-1}$ (lit.,[4] m.p. 151°, $[\alpha]_D$ +111°).

Desacetyldihydroisotenulin.—A. Desacetyldihydroisotenulin was obtained in 38% yield using the method of Barton and deMayo[4], and had m.p. 202°; $[\alpha]_D$ +156° ($CHCl_3$); infrared: 3540, 1760, 1740 cm.$^{-1}$ (lit.,[4] m.p. 203°; $[\alpha]_D$ +150.7°; infrared: 3500, 1770, 1732 cm.$^{-1}$).

B. Desacetylisotenulin, isolated from the plant material on alumina columns, would not absorb hydrogen in ethanol at atmospheric pressure using either Adams' catalyst or 5% palladium-on-carbon. Hydrogenation was successfully completed on the Parr apparatus (20 p.s.i. for 48 hr.). Work-up of the reaction mixture and recrystallization from ethyl

(25) W. T. Smith, Jr., and R. L. Shriner, "The Examination of New Organic Compounds," John Wiley & Sons, Inc., New York, N. Y. 1956.

(26) T. A. Geissman and P. Deuel, *J. Am. Chem. Soc.*, **79**, 3778 (1957).

(27) W. M. Schubert and H. Burkett, *ibid.*, **78**, 64 (1956).

acetate–petroleum ether produced desacetyldihydroisotenulin; it had m.p. 196–198°; a mixed melting point with authentic material was 199–202°; infrared: 3520, 1760, 1740 cm.$^{-1}$.

Dehydrodesacetyldihydroisotenulin.—The method of Herz and Mitra[9] was used. Needles (30%) were obtained from ethyl acetate–petroleum ether: m.p. 146–147°; infrared: 1755–1760, 1730, 1697 cm.$^{-1}$ (lit.,[9] m.p. 144–146°; infrared: 1778, 1758, 1710 cm.$^{-1}$).

N.m.r. spectra were obtained at 60 Mc. in deuteriochloroform, with tetramethylsilane as internal standard. The following tabulation describes the peaks observed, in cycles per second.

Bigelovin: 72, singlet, 3H (C-5 methyl); 73, 83, doublet, 3H (C-10 methyl); 87–108, multiplet (CH_2, CH); 118, singlet, 3H (acetyl methyl); 177–200, multiplet (allylic C-1, C-7 protons); 268, 271, 278, 281, 290, 293, split triplet, 1H (C-8 proton); 334, 342, doublet, 1H (C-6 proton); 364, 367, 370, 373, split doublet, 1H (C-3 proton); 354, 357, 372, 375, split doublet, 2H (C-13 protons, exocyclic methylene); 469, 472, 463, 465, split doublet, 1H (C-2 proton).

Dihydrobigelovin[28]: 63, 64, 68, 70, 73, multiplet (methyl); 65, singlet, 3H (C-5 methyl); 77–106, multiplet (CH_2, CH); 118, singlet, 3H (acetyl methyl); 170–195, 133–158, multiplets (protons at C-3, C-7); 260–263, 271, 274, 281, 284, split triplet, 1H (C-8 proton); 327, 335, 317, 326, two doublets (C-6 proton); 351, 354, 372, 375, split doublet, 2H (C-13 protons, exocyclic methylene).

Tetrahydrobigelovin: 63, 67, 74, multiplet (methyl); 66, singlet, 3H (C-5 methyl); 80–112, multiplet (CH_2, CH); 117, singlet, 3H (acetyl methyl); 130–170, multiplet (C-3, C-11 protons); 260, 262, 270, 272, 282, 284, split triplet, 1H (C-8 proton); 318, 316, doublet, 1H (C-6 proton).

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(28) Some contamination with the tetrahydro compound, as revealed by comparison of the n.m.r. spectra. The data are gived to show the absence of the C-2 and C-3 vinyl protons and the presence of the exocyclic methylene group.

Angularine, a New Pyrrolizidine Alkaloid from *Senecio angulatus* L.

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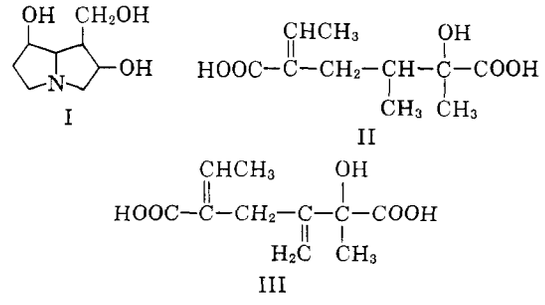
Rosmarinine, the senecic acid ester of rosmarinecine, and a new alkaloid, angularine, the corresponding seneciphyllic acid ester, have been isolated from *Senecio angulatus* L.

Assays of a number of hitherto unexamined species of the genus *Senecio* led to the observation that *Senecio angulatus* L., an ornamental plant native to South Africa and cultivated in Southern California, yields up to 1.5% (dry basis) of crystalline alkaloid. The crude alkaloid, isolated by the usual methods, showed variable melting point behavior from one preparation to another, and gave analytical results that agreed with a molecular formula within the limits of $C_{18}H_{25-27}NO_6$. On paper chromatograms the substance showed an elongated spot which, by comparison with the well defined spots given under the same conditions by specimens of other, known, *Senecio* alkaloids, appeared to consist of two components.

Hydrolysis of the unresolved alkaloid mixture gave the known pyrrolizidine base, rosmarinecine (I),[1,2] identified by comparison with an authentic specimen and the preparation of its triacetate picrate, dibenzoate, anhydro base picrate, and anhydro base acetate picrate. These were identified by their properties and by direct comparison with authentic specimens.[3]

The other product from the hydrolysis of the alkaloid mixture was a crystalline acid that was at first regarded as a single compound and which was at length found to be a mixture. Its melting point was 114–116° when first isolated, but this altered upon repeated recrystallization, eventually broadening to about 116–129°. Separation of the mixture into senecic (II) and seneciphyllic (III) acids was achieved by partition chromatography on a silicic acid column.



I

II

III

Comparison of the infrared absorption spectrum of a mixture of equal parts of senecic and seneciphyllic acids, with that of the crude mixture of acids obtained from the unresolved alkaloid gave an indication that the plant contains roughly equal amounts of the corresponding alkaloids. The n.m.r. spectrum of the methyl esters of the acid mixture could be interpreted on the same grounds: the methyl groups of the ester groupings gave sharp 3H

(1) M. Richardson and F. L. Warren, *J. Chem. Soc.*, 452 (1943).

(2) L. J. Dry, M. J. Koerkemoer, and F. L. Warren, *ibid.*, 59 (1955).

(3) We are grateful to Prof. F. L. Warren for specimens of rosmarinecine and the picrate of tri-O-acetylrosmarinecine.