Constituents of *Helenium* Species. XI. The Structure of Pinnatifidin[1,2]

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The structure of pinnatifidin, a sesquiterpene lactone from *Helenium pinnatifidum* (Nutt.) Rybd., has been established.

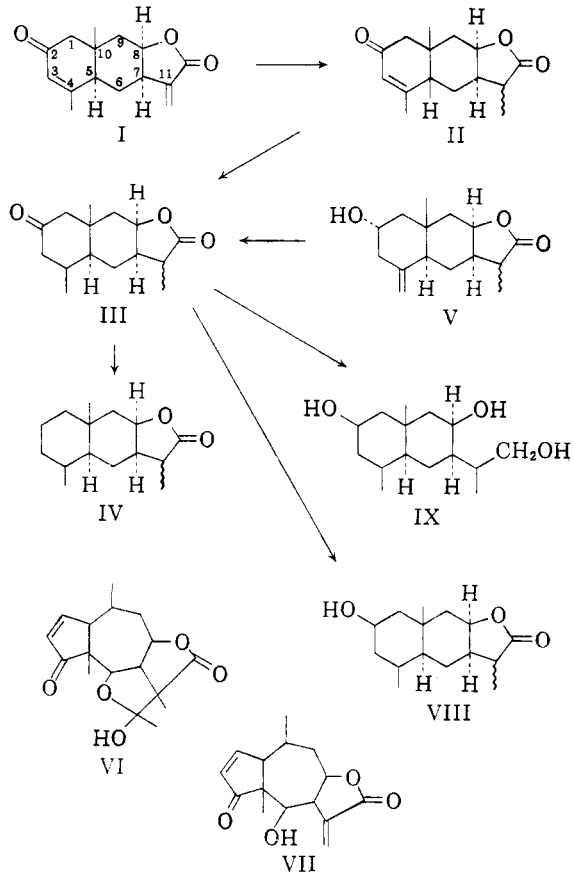
The isolation of pinnatifidin, a new sesquiterpene lactone of formula $C_{15}H_{18}O_3$, from the herb of *Helenium pinnatifidum* (Nutt.) Rydb.. has been reported previously.[4] We now show that its structure is represented by I.

The ultraviolet spectrum of pinnatifidin, λ_{max} 237.5 mμ (ϵ 13,550), indicated the presence of a disubstituted α,β-unsaturated ketone. This was supported by the infrared spectrum which exhibited a strong band at 1675 cm.$^{-1}$ characteristic of a conjugated ketone group not in a five-membered ring. The remaining two oxygen atoms were presumably present as a γ-lactone (infrared band at 1770 cm.$^{-1}$) conjugated with a methylene group (high intensity at 210 mμ, band at 1.64μ in the near infrared,[5] strong band at 1630 cm.$^{-1}$).

The presence of an exocyclic methylene group conjugated with a lactone was shown chemically by ozonolysis (formation of formaldehyde), preparation of a pyrazoline and partial reduction (Lindlar catalyst) to dihydropinnatifidin (II). This substance still retained the α,β-unsaturated ketone chromophore, λ_{max} 237 mμ (ϵ 10,500), infrared bands at 1768 (γ-lactone), 1665 (conjugated carbonyl) and 1620 cm.$^{-1}$ (conjugated double bond), but no longer exhibited high intensity absorption at 210 mμ and liberated no formaldehyde on ozonolysis. Instead, there was formed formic acid and a gummy keto acid which suggested the presence of the partial structure —C(=O)—CH=CR_2 as part of a ring.

Confirmation for this was found in the properties of tetrahydropinnatifidin (III) which resulted from the saturation of both double bonds. The ketone group of this substance appeared to be in a six-membered ring (infrared band at 1715 cm.$^{-1}$) and was flanked by a methylene group (band at 1430 cm.$^{-1}$, positive Zimmermann test, condensation with piperonal).

The n.m.r. spectra[6] of I, II, and III verified these conclusions. I had two low-field doublets at 6.09 and 5.59 p.p.m., J = 1.5 c.p.s., each representing one proton characteristic of the methylene group conjugated with a lactone carbonyl.[1,7,8] These were absent in II and III. A vinyl proton singlet, somewhat broadened by long range spin coupling, was found in the spectra of I and II, but not in the spectrum of III. Its chemical shift (5.83 p.p.m.) is characteristic of a proton alpha to a carbonyl in an α,β-unsaturated ketone.



Pinnatifidin had two sharp signals characteristic of methyl groups. One of these was at 1.00 p.p.m. (tertiary methyl); II and III exhibited this singlet at 0.88 and 0.97 p.p.m. and had a new methyl doublet at 1.13 and 1.21 p.p.m. respectively (J = 7, methyl alpha to lactone). The second

(1) Previous paper, W. Herz, W. A. Rohde, K. Rabindran, P. Jayaraman, and N. Viswanathan, *J. Am. Chem. Soc.*, **84**, 3857 (1962).

(2) Supported in part by grants from the National Science Foundation (NSF-G 14396) and the United States Public Health Service (RG-5814).

(3) Recipient of a Fulbright Travel Award, 1957–1958.

(4) W. Herz, R. B. Mitra, K. Rabindran, and W. A. Rohde, *J. Am. Chem. Soc.*, **81**, 1481 (1959).

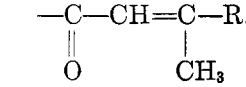
(5) W. H. Washburn and M. S. Mahoney, *ibid.*, **90**, 504 (1958).

(6) Spectra were run by Mr. Fred Boerwinkle in deuteriochloroform solution at 60 Mc. on a Varian HR-60 instrument, with tetramethylsilane as internal standard. Frequencies were determined by the side band technique. Funds for the purchase of the spectrometer were provided by the Institute of Molecular Biophysics of the Florida State University.

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

(8) W. Herz and G. Högenauer, *J. Org. Chem.*, **27**, 905 (1962).

methyl singlet of pinnatifidin had a chemical shift characteristic of a vinyl methyl group (1.95 p.p.m.). Dihydropinnatifidin retained this signal at 1.80 p.p.m., but it was lacking in tetrahydropinnatifidin which instead exhibited an additional methyl doublet at 0.92 p.p.m. Hence the chromophore of pinnatifidin can be expanded to



—C—CH=C—R.[9]

Conversion of II to the thioketal followed by Raney nickel desulfurization resulted in the formation of tetrahydroalantolactone (IV), identical in all respects with an authentic sample.[10] Consideration of the evidence presented in the preceding paragraphs therefore requires that pinnatifidin be represented by I. This is surprising indeed because all other sesquiterpene lactones from *Helenium* species which we have studied appear to possess the "abnormal" carbon skeleton of tenulin (VI)[1] and helenalin (VII).[11] It remains to be seen whether this findings possess taxonomic significance.

The structure of tetrahydropinnatifidin (III), m.p. 203–204°, $[\alpha]^{23}D$ +71.3° is identical with the structure previously[8] deduced for dehydrotetrahydroivalin, a transformation product of ivalin, for which we reported m.p. 195–197°, $[\alpha]^{29}D$ +73.2°. Careful purification of a sample of dehydrotetrahydroivalin raised the m.p. to 201–204°. There was a slight depression to 198–200° on admixture of the two samples, but comparison of rotations, infrared spectra and mobilities on a thin-layer chromatogram showed that tetrahydropinnatifidin and dehydrotetraivalin were indeed identical.

Additional evidence was provided by the sodium borohydride reduction of III which furnished material identical in all respects with 2-epitetrahydroivalin (VIII).[8] Lithium aluminum hydride reduction of III gave a triol (IX) epimeric at C-2 with the triol[8] from tetrahydroivalin.

Experimental[12]

Pinnatifidin.—Pinnatifidin was isolated in the manner described earlier[4] from dried *Helenium pinnatifidum* (Nutt.) Rydb., collected in late April and early May 1956, 1957, and 1958, near St. Marks, Florida: average yield 0.12% of material melting in the range 161–164°. The Zimmermann test was negative, presumably because of hindrance at C-1.

A solution of 0.05 g. of pinnatifidin and 0.05 g. of piperonal in 2 ml. of absolute ethanol was mixed with 3 ml. of ethanol saturated with dry hydrogen chloride in the cold and kept at 0° overnight. Dilution with water furnished yellow needles which were recrystallized from ethanol, yield 0.065 g., m.p. 239°. The analysis indicated that condensation had proceeded only after initial addition of hydrogen chloride (positive halogen test).

Anal. Calcd. for $C_{24}H_{23}O_5Cl$: C, 66.59; H, 5.55. Found: C, 66.82; H, 5.52.

The pyrazoline was prepared by mixing a saturated solution of 0.1 g. of pinnatifidin in ether with 10 ml. of an ethereal solution of diazomethane in ether and keeping in the refrigerator for 2 days. The product, wt. 0.08 g., was filtered and recrystallized from benzene–hexane, m.p. 152–153°.

Anal. Calcd. for $C_{16}H_{20}N_2O_3$: C, 66.64; H, 6.99; N, 9.72. Found: C, 66.41; H, 6.82; N, 9.45.

A solution of 0.082 g. of pinnatifidin in 10 ml. of acetic acid was ozonized for 25 min. at room temperature, diluted with 10 ml. of water and steam distilled into a chilled solution of dimedone in water. After standing there precipitated 29 mg. (49%) of the dimedone derivative of formaldehyde, m.p. 183–185°.

Dihydropinnatifidin (II).—A suspension of 0.15 g. of Lindlar catalyst in 50 ml. of ethyl acetate was saturated with hydrogen, mixed with 0.5 g. of pinnatifidin and the hydrogenation continued at atmospheric pressure. Hydrogen absorption stopped after consumption of one mole-equivalent of hydrogen. Removal of solvent yielded crystalline II in quantitative yield, m.p. 186–188°. Recrystallization from benzene–petroleum ether raised the m.p. to 189.5–190.5°, $[\alpha]^{23}D$ + 132.3° (95% ethanol, *c*, 0.47).

Anal. Calcd. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12; C-methyl, 2.0. Found: C, 72.51; H, 8.16; C-methyl, 1.2.

A solution of 0.15 g. of II in 25 ml. of chloroform was ozonized for 30 min. at 0°. The mixture was added to 30 ml. of water and the combined solvents distilled into a chilled receiver. The two-phase distillate was neutralized by titration with 0.02 *N* sodium hydroxide solution; consumption corresponded to 25 mg. of formic acid (90%). Solvents were removed and the residue converted to the *p*-bromophenacyl ester, yield 15 mg. of *p*-bromophenacyl formate, m.p. 139–140°, undepressed on admixture of an authentic sample. The nonvolatile residue of the ozonolysis was an acidic gum which gave a positive test with dinitrophenylhydrazine.

Tetrahydropinnatifidin (III).—Hydrogenation of 0.107 g. of pinnatifidin in 25 ml. of ethyl acetate with 10 mg. of platinum oxide in a semimicro hydrogenation apparatus resulted in the uptake of 19.8 ml. of hydrogen (calcd. for two double bonds 19.1 ml.). Removal of solvent resulted in a quantitative yield of III, m.p. 195–200°, which recrystallization from benzene-petroleum ether (b.p. 60–90°) raised to 203–204°, $[\alpha]^{22}D$ +71.3° (95% ethanol, *c*, 0.35). The substance gave a positive Zimmermann test.

Anal. Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 72.21; H, 8.77.

A sample of dehydrotetrahydroivalin[8] was recrystallized repeatedly from benzene–petroleum ether, m.p. 201–204°, mixed m.p. with III, 198–200°, infrared spectra superimposable. Reduction of III with sodium borohydride in the manner described previously gave 2-epitetrahydroivalin (VIII),[8] m.p. 169–170°, undepressed on admixture of an authentic sample.

The semicarbazone of III was prepared by refluxing 0.1 g. of III with 0.1 g. of semicarbazide hydrochloride and 0.2 g. of sodium acetate in 2 ml. of aqueous methanol, yield 0.095 g., m.p. 237–238°.

Anal. Calcd. for $C_{16}H_{25}N_3O_3$: C, 62.52; H, 8.20; N, 13.7. Found: C, 61.73; H, 8.46; N, 14.0.

The piperonylidene derivative, 85 mg. of product from 55 mg. of III, melted at 253–254° after recrystallization from alcohol. It retained solvent of crystallization.

Anal. Calcd. for $C_{23}H_{26}O_5 \cdot {}^1/_2C_2H_6O$: C, 71.11; H, 7.16. Found: C, 71.31; H, 7.00.

(9) All spectra also exhibited a complex band centered at 4.50 p.p.m. due to hydrogen on carbon carrying the lactone oxygen.

(10) We wish to thank Dr. K. Tanabe for supplying this material. See ref. 8, footnotes 12 and 13 for literature references and comment on stereochemistry at C-11.

(11) W. Herz, A. Romode Vivar, J. Romo, and N. Viswanathan, *J. Am. Chem Soc.*, in press.

(12) Melting points are uncorrected. Analyses by Dr. Weiler, and Strauss, Oxford, England, and Dr. F. Pascher, Bonn, Germany. Ultraviolet spectra were run in 95% ethanol solution. Infrared spectra were run in chloroform solution.

Vigorous drying at 140° resulted in loss of solvent of crystallization.

Anal. Calcd. for $C_{23}H_{26}O_5$: C, 72.23; H, 6.85. Found: C, 72.47; H, 6.83.

Tetrahydroalantolactone (IV).—A mixture of 0.2 g. of III, 0.5 ml. of ethanedithiol and 0.5 ml. of boron trifluoride etherate was allowed to stand overnight, diluted with water, and extracted with ether. The ether extracts were washed, dried, and concentrated; yield 0.24 g. of thioketal, m.p. 160°, which recrystallization from benzene–petroleum ether raised to 165–166°.

Anal. Calcd. for $C_{17}H_{26}O_2S_2$: C, 62.82; H, 8.05; S, 19.6. Found: C, 62.56; H, 8.03; S, 19.6.

A solution of 0.25 g. of the thioketal in 25 ml. of ethanol was mixed with 1 teaspoonful of Raney nickel and refluxed with stirring for 25 hr. On working up in the usual manner, there was obtained after recrystallization 0.05 g. of tetrahydroalantolactone, m.p. 142°, undepressed on admixture of an authentic sample of m.p. 142°, infrared spectra superimposable.

Lithium Aluminum Hydride Reduction of Tetrahydropinnatifidin.—Reduction of 1 g. of III with 0.5 g. of lithium aluminum hydride in anhydrous ether by the Soxhlet method, decomposition of excess hydride with water and dilute sulfuric acid, washing and drying of the ether layer yielded on concentration 0.8 g. of IX, m.p. 190°. Recrystallization from benzene–methanol raised the m.p. to 191–192°, $[\alpha]^{25}D$ +25.8° (95% ethanol, *c*, 0.946).

Anal. Calcd. for $C_{15}H_{28}O_3$: C, 70.27; H, 11.01. Found: C, 70.05; H, 11.00.

Constituents of *Helenium* Species. XII. Sesquiterpene Lactones of Some Southwestern Species[1,2]

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Tenulin, isotenulin, helenalin, neohelenalin, mexicanin E, and two new sesquiterpene lactones linifolin A and linifolin B were isolated in a study of six not previously investigated *Helenium* species. Structures for the new compounds are proposed.

The genus *Helenium* is rich in sesquiterpene lactones. Helenalin (I)[3] and tenulin (II),[4] the main constituents of *H. autumnale* L.[5] and *H. amarum* (Raf.) H. Rock,[6] respectively, also have been isolated from the Southwestern species *H. microcephalum* DC.,[8] *H. elegans* DC.,[5,9] and *H. mexicanum* H.B.K.[10] and from some members of the vernal-flowering Southeastern section *Leptopoda*.[7,11,12] A series of new sesquiterpene lactones has been obtained from Southeastern species,[2,11,12] from *H. mexicanum* H.B.K.[3,10,13] and from *H. bigelovii* Gray.[15] We now wish to report the results of an investigation of six Southwestern species which have not been examined previously. The remaining member of the section *Leptopoda* has also been studied.

All Southwestern species contained relatively large amounts of a sesquiterpene lactone fraction, but the yields of crystalline substances were generally small. *Helenium arizonicum* Blake afforded isotenulin (III).[16] *H. laciniatum* Gray yielded helenalin. *H. scorzoneraefolium* (DC.) Gray gave an isomer of helenalin, m.p. 149–150°; the amount isolated was too small to permit further investigation. *H. Bloomquistii* Rock[17] yielded tenulin.

Extraction of *H. linifolium* Rydb. and extensive chromatography furnished tenulin and two previously undescribed substances of formula $C_{17}H_{20}O_5$ which were named linifolin A and linifolin B. Linifolin A, m.p. 195–198°, $[\alpha]^{25}D$ +30°, was assigned structure IV on the basis of the following evidence.

The ultraviolet spectrum, λ_{max} 215 and 320–325 mμ (ε12,300 and 43) was similar to that of helenalin[4] and many of its congeners, the absorption being due to superposition of the cyclopentenone chromophore (infrared bands at 1710 and 1595 cm.$^{-1}$) and the α, β-unsaturated lactone (infrared bands at 1755—double strength, combination of γ-lactone and acetate—and 1660 cm.$^{-1}$). The presence of the

(1) Supported in part by a grant from the U.S. Public Health Service (RG-5814).

(2) Previous paper, W. Herz, R. B. Mitra, K. Rabindran, and N. Viswanathan, *J. Org. Chem.*, **27**, 4041 (1962).

(3) W. Herz, A. Romo de Vivar, J. Romo, and N. Viswanathan, *J. Am. Chem. Soc.*, in press.

(4) W. Herz, W. A. Rohde, K. Rabindran, P. Jayaraman, and N. Viswanathan, *ibid.*, **84**, 3851 (1962).

(5) E. P. Clark, *ibid.*, **58**, 1982 (1936).

(6) E. P. Clark, *ibid.*, **61**, 1836 (1939); **62**, 597 (1940). This is the prior designation for the more commonly employed *H. tenuifolium* Nutt.[7]

(7) H. F. L. Rock, *Rhodora*, **59**, 101, 128, 168, 203 (1957).

(8) R. Adams and W. Herz, *J. Am. Chem. Soc.*, **71**, 2546 (1949).

(9) The possible conspecificity of *H. montanum* Nutt., *H. quadridentatum* Labill., and *H. badium* (Gray) Greene, also investigated by Clark,[8] with one of the aforementioned species is now being studied by Dr. H. F. L. Rock (private communication). *H. macrocephalum* mentioned by Clark is undoubtedly a misprint for *H. microcephalum*, since no such species is recorded in the botanical literature.

(10) A. Romo de Vivar and J. Romo, *Chem. Ind.* (London), 882 (1959); *Ciencia* (Mex.), **21**, (1), 33 (1961).

(11) W. Herz, R. B. Mitra, K. Rabindran, and W. A. Rohde, *J. Am. Chem. Soc.*, **81**, 1481 (1959).

(12) W. Herz, P. Jayaraman, and H. Watanabe, *ibid.*, **82**, 2276 (1960).

(13) This includes the interesting norsesquiterpene lactone mexicanin E[14] (*vide infra*).

(14) A. Romo de Vivar and J. Romo, *J. Am. Chem. Soc.*, **83**, 2326 (1961).

(15) Private communication from Professor T. A. Geissman University of California at Los Angeles.

(16) Presumably this is not an artifact resulting from isomerization of tenulin during the isolation procedure, since other species (*vide infra*) yielded tenulin and no isotenulin.

(17) This is a new species (private communication from Dr. Rock) whose description has not yet been published.