

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE FLORIDA STATE UNIVERSITY]

Constituents of Helenium Species. IX. The Sesquiterpene Lactones of *H. flexuosum* Raf. and *H. campestre* Small¹

BY WERNER HERZ, P. JAYARAMAN AND HIROSHI WATANABE²

RECEIVED OCTOBER 15, 1959

A substance $C_{15}H_{18}O_4$ previously isolated from *H. flexuosum* Raf. has been identified as neohelenalin. Later extractions of this plant did not give neohelenalin but yielded two new lactones of formula $C_{17}H_{24}O_6$. Helenalin was isolated from *H. campestre* Small.

In an earlier paper³ we reported the isolation of new sesquiterpene lactones from several representatives of the genus *Helenium*, subgenus *Leptopoda*. Included in this group was a substance $C_{15}H_{18}O_4$ obtained in small quantity by chromatographing the crude extract of *H. flexuosum* Raf. which appeared to be new and was named flexuosin.

On chromatographing a crude helenalin extract from *Balduina angustifolia* (Pursh) Robins⁴ over basic alumina, it was noticed that partial isomerization appeared to take place on the column. The yield of helenalin (I)⁵ was reduced greatly and resinification was extensive. From the more polar fractions there was isolated a substance of m.p. 225–226°, $[\alpha]^{24}_D$ 143°, whose infrared spectrum was identical in all respects with the spectrum of flexuosin. The identity of the two samples was further established by a mixed m.p. determination and a comparison of the ultraviolet spectra. The somewhat lower m.p. and rotation of the material from *H. flexuosum*³ must be ascribed to the presence of a small amount of contaminant.

The following evidence indicates that this compound has structure II and should therefore be called neohelenalin,^{6–8} the name flexuosin being reserved for other extractives of *H. flexuosum* (*vide infra*). The infrared spectrum (rel. strong band at 1640 cm^{-1}) suggested that the substance, unlike helenalin, had only one double bond. However, this band remained unaffected by catalytic hydrogenation which consumed one mole of hydrogen and led to dihydroneohelenalin (III). The latter also retained the band at 1695 cm^{-1} previously ascribed to a cyclopentenone group; its ultraviolet spectrum is consonant with that of a tetrasubstituted cyclopentenone and closely resembles that of desacetylneotenulin.^{7,9} The ultraviolet spectrum of dihydroneohelenalin differs from the ultraviolet spectrum of neohelenalin³ in the disappearance of

high-intensity absorption in the 210–220 $m\mu$ region which would be expected if the exocyclic methylene group of helenalin and II, also responsible for the near-infrared band at 1.64 μ ,¹¹ were reduced on hydrogenation. Ozonolysis corroborated these conclusions. Neohelenalin yielded formaldehyde and acetic acid, the latter arising by degradation of the α -methyl- α,β -unsaturated ketone. Ozonolysis of dihydroneohelenalin furnished acetic acid only. The shape of the optical rotatory dispersion curve of dihydroneohelenalin parallels that of desacetylneotenulin,¹² although the amplitude is smaller which indicates that the stereochemistry at C_1 is the same in both compounds.

These results establish the site and nature of the chromophoric groups present in neohelenalin. There is as yet no direct evidence for the orientation of the lactone ring which is provisionally closed toward C_8 as deduced by Büchi and Rosenthal⁵ for the parent compound on the basis of n.m.r. spectra and not toward C_6 as in IV, since relactonization on the column seemed not very likely. Several attempts to place this assumption on a somewhat more secure footing failed and are recorded in the Experimental part. Of some interest was our failure to obtain a mesylate of neohelenalin. In contrast, helenalin and tetrahydrohelenalin gave mesylates whose behavior when subjected to the elimination reaction is worthy of comment. Tetrahydrohelenalin mesylate on treatment with lutidine gave an α,β -unsaturated lactone to which, on the basis of Büchi and Rosenthal's formula,⁵ structure V must be assigned (λ max 218, 291 $m\mu$, ϵ max 15600, 38, infrared bands at 1735 and 1670 cm^{-1} , the latter unusually strong). On the other hand the substance obtained in poor yield by base treatment of helenalin mesylate, while containing an additional element of unsaturation, (three $C=C$ stretching frequencies at 1660, 1595 and 1570 cm^{-1}) was not the expected trienone VI whose formation would have provided chemical confirmation for the postulated structure of helenalin. Instead its ultraviolet spectrum (λ max 221 $m\mu$, ϵ max 10900, high extinction coefficient at 210 $m\mu$) was similar to that of helenalin and indicated that the newly-introduced double bond was not in conjugation with either of the two chromophores present in I, unless cross-conjugated structures such as VIIa and VIIb present some unusual features.¹³

(1) Previous paper, W. Herz, R. B. Mitra and P. Jayaraman, *THIS JOURNAL*, **81**, 6061 (1959).

(2) Recipient of a Fulbright Travel Award, 1958–1959.

(3) W. Herz, R. B. Mitra, K. Rabindran and W. A. Rohde, *THIS JOURNAL*, **81**, 1481 (1959).

(4) W. Herz and R. B. Mitra, *ibid.*, **80**, 4876 (1958).

(5) G. Büchi and D. Rosenthal, *ibid.*, **78**, 3860 (1956).

(6) The prefix neo is used in the sense previously applied to desacetylneotenulin.¹

(7) D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 142 (1956).

(8) Professor G. Büchi has informed us that he has also observed the conversion of helenalin to neohelenalin on a basic alumina column and has reached the same conclusion regarding its structure. Reference to dihydroneohelenalin, without details, is made in an article by L. Bernardi and G. Büchi, *Experientia*, **13**, 466 (1957).

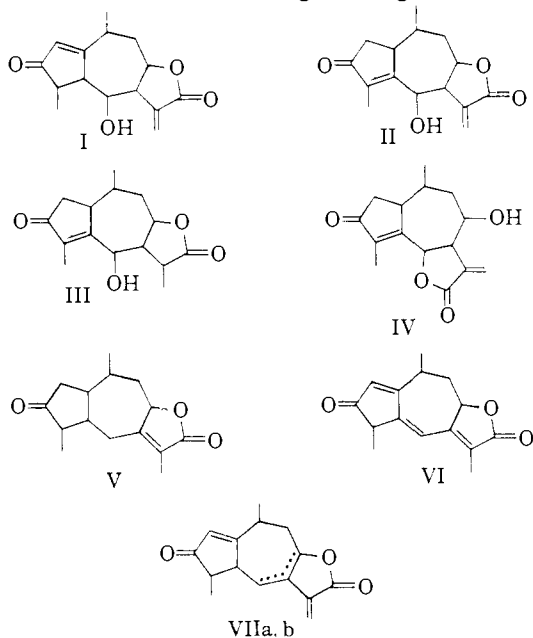
(9) The tetrasubstituted double bond of desacetylneotenulin is also resistant to hydrogenation.¹⁰

(10) W. Herz, K. Rabindran and W. A. Rohde, to be published.

(11) W. H. Washburn and M. J. Mahoney, *THIS JOURNAL*, **80**, 504 (1958).

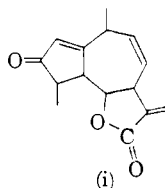
(12) C. Djerassi, J. Osiecki and W. Herz, *J. Org. Chem.*, **22**, 1361 (1957). We wish to thank Professor Djerassi and Dr. E. J. Eisenbraun for carrying out this measurement. Some difficulty was experienced due to the low solubility of dihydroneohelenalin.

In view of the experience thus gained with *B. angustifolia*, it seemed possible that the neohelenalin previously isolated from *H. flexuosum* which represented but a small portion of the total extract was an artifact or at most a minor constituent. Renewed examination of plant material collected at several widely separated locales, including the one from which the original sample of *H. flexuosum* had been obtained, seemed to bear this out. Each extraction furnished two apparently new isomeric lactones of formula $C_{17}H_{24}O_6$, but no neohelenalin.¹⁴ Isolated in larger quantity (0.2% yield from a large-scale extraction) was a compound which we have named flexuosin A, m.p. 220–221.5°, (α)_D 12.4°. A less polar lactone, flexuosin B, was obtained in 0.09% yield and had m.p. 132–137°, (α)_D 44.2. Flexuosin A exhibited only end absorption in the ultraviolet (ϵ 8750 at 210 $m\mu$). Flexuosin B had λ max 218 and 290 $m\mu$ (ϵ 12500 and 30). The structure of these substances is now being investigated.



Through the courtesy of Dr. B. H. Braun we also came into possession of a supply of *H. campestre*

(13) A referee has commented that this dehydration appears to afford good evidence for the introduction of a disubstituted ethylenic linkage and for the formulation of VII as (i). We have also considered this which, unless the elimination reaction were accompanied by a rearrangement, would imply a reversal of the helenalin lactone orientation postulated by Büchi and Rosenthal⁵ and would render necessary reconsideration of conclusions reached earlier⁴ on the basis of this postulate. The low yield of VII and inadequate supply of helenalin precluded further investigation at this time but additional work on helenalin is obviously desirable.



(14) Our failure to isolate neohelenalin may also be the result of seasonal variation in the constituents of the plant.

(15) The coincidence in m.p. with our earlier³ somewhat impure sample of neohelenalin is fortuitous.

pestre Small, one of the two members of the subgenus *Leptopoda* which had not been previously³ investigated. Extraction of this species whose distribution is severely limited¹⁶ furnished helenalin as the sole crystallizable constituent.

Acknowledgment.—This work was supported in part by grants from the National Science Foundation (G-3313) and the United States Public Health Service (RG-5814). We are grateful to the Research Council of the Florida State University for funds toward the purchase of an extractor. Special thanks are due Drs. Robert K. Godfrey of our Department of Botany, H. F. L. Rock of the University of Tennessee, and B. H. Braun for help with the collection and identification of *H. flexuosum* and to Dr. Braun for collecting *H. campestre*.

Experimental¹⁷

Neohelenalin.—A crude extract of *B. angustifolia* (Pursh) Robins., (collected at various locations in West Florida in August and September, 1958), wt. 200 g., was dissolved in 500 ml. of benzene and chromatographed in the way described earlier⁴ over basic alumina (Alcoa grade F-20). Considerable decomposition seemed to take place. Elution with benzene furnished only 4.2 g. of helenalin, m.p. 163–165°. Elution with benzene-ethanol (19:1) gave 5 g. of neohelenalin, m.p. 219–220°, raised to 225° on recrystallization from benzene. Later fractions could not be induced to solidify.

Neohelenalin on rechromatography was shown to be a homogeneous substance, m.p. 225–226°, (α)_D 143° (95% ethanol, c 0.335). A mixed m.p. with "flexuosin," m.p. 221°, (α)_D 90° (the sign of the rotation given in ref. 3 was erroneously reversed) was 221–225°. The infrared spectra of the two samples were superimposable, major bands at 3400 (hydroxyl), 1760 (γ -lactone), 1695 and 1640 (cyclopentenone) and 1410 cm^{-1} λ_{max} 235 and 208 $m\mu$ (ϵ 17800 and 13800).

Anal. Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.19; H, 6.73.

Ozonolysis of Neohelenalin.—(A) A solution of 120 mg. of neohelenalin in 20 ml. of acetic acid was ozonized at room temperature for 45 minutes, diluted with water and steam distilled into 200 ml. of dimedone solution. On standing there precipitated 30 mg. (23%) of the dimedone derivative of formaldehyde, m.p. 180–181°, undepressed on admixture of an authentic sample.

(B) A solution of 120 mg. of neohelenalin in 20 ml. of chloroform was ozonized for 1 hr. at 0°, diluted with 20 ml. of water and steam-distilled. The distillate, 300 ml., was made alkaline to phenolphthalein, and the chloroform drawn off. The aqueous layer was neutralized and refluxed for 1 hr. with 5 ml. of *p*-bromophenacyl bromide in 5 ml. of ethanol. Removal of solvent *in vacuo* precipitated 75 mg. of crude *p*-bromophenacyl acetate, m.p. 65–70°. On chromatography the m.p. was raised to 82°, undepressed on admixture of an authentic sample.

Dihydroneohelenalin.—A solution of 100 mg. of neohelenalin in 50 ml. of ethanol was reduced with 10 mg. of pre-reduced platinum oxide. The theoretical quantity of hydrogen was absorbed in 1 hr. Removal of solvent followed by two crystallizations from ethanol yielded dihydroneohelenalin, m.p. 270° (dec.), (α)_D -34° (95% ethanol, c 0.11), λ_{max} 238 and 294–296 $m\mu$ (ϵ 15600 and 104). The infrared spectrum (Nujol mull) had bands at 1755 (γ -lactone), 1695 and 1632 cm^{-1} (cyclopentenone).

Anal. Calcd. for $C_{15}H_{20}O_4$; C, 68.16; H, 7.62. Found: C, 68.00; H, 7.68.

Treatment of dihydroneohelenalin with acetic anhydride and sodium acetate gave an oil, wt. 60 mg., which was chromatographed over acid-washed alumina, solvent and eluent benzene. The major band yielded semisolid material which was recrystallized from benzene-petroleum

(16) H. F. L. Rock, *Rhodora*, **59**, 101, 128, 159 (1957).

(17) M.p.'s and b.p.'s are uncorrected. Analyses by Drs. Weiler and Strauss, Oxford. Infrared spectra were run in chloroform solution and ultraviolet spectra in 95% ethanol, unless otherwise specified.

ether. The acetate, silky white needles, m.p. 150–151°, (α)²⁵D –213° (95% ethanol, C, 0.26) had infrared bands at 1760 (γ -lactone), 1730 (acetate) 1695 and 1630 cm.⁻¹ (cyclopentenone). Acetylation of dihydroneohelenalin with pyridine-acetic anhydride was unsuccessful.

Anal. Calcd. for C₁₇H₂₂O₆: C, 66.64; H, 7.24. Found: C, 66.90; H, 7.39.

Ozonolysis of dihydroneohelenalin gave no formaldehyde, but only acetic acid identified as the *p*-bromophenacyl ester.

An attempt to oxidize dihydroneohelenalin in the manner reported for desacetylneotenulin⁷ failed. Although chromic acid was consumed, 45% of starting material was recovered by dilution with water and workup of the chloroform extract yielded an additional 15% of dihydroneohelenalin.

Treatment of dihydroneohelenalin with the equivalent amount of methanesulfonyl chloride in pyridine gave a gum whose infrared spectrum was virtually identical with that of starting material. Use of excess mesyl chloride resulted in a mixture which on chromatography gave a 25% recovery of starting material and gums which could not be crystallized. Distillation of the latter in a high vacuum gave no homogeneous material.

Helenalin Mesylate.—A mixture of 1.3 g. of helenalin, 11 ml. of dry pyridine and 1.5 ml. of methanesulfonyl chloride was kept in the refrigerator overnight and then poured into ice-hydrochloric acid. Extraction with chloroform and purification in the usual manner yielded an oil, wt. 1.45 g., which was dissolved in benzene and chromatographed over 20 g. of acid-washed alumina. Benzene eluted the product which crystallized on standing, wt. 0.71 g. Recrystallization from benzene-petroleum ether gave a constant m.p. of 144°.

Anal. Calcd. for C₁₆H₂₀O₆S: C, 56.44; H, 5.92, S, 9.41. Found: C, 56.62; H, 6.01; S, 9.25.

A mixt. of 0.70 g. of the above and 15 ml. of 2,6-lutidine was refluxed for 24 hr., cooled and poured into ice-hydrochloric acid. Extraction with chloroform and purification in the usual manner yielded a resin, wt. 0.78 g., which was dissolved in 10 ml. of benzene and chromatographed over 10 g. of alumina. The fraction eluted with benzene-5% ether crystallized on standing, wt. 0.12 g. Recrystallization from benzene-petroleum ether gave needles which sintered near 120° but did not decompose below 260°, (α)²⁵D –100° (95% ethanol, C, 0.115), λ_{\max} 221 m μ , ϵ 10900, infrared bands at 1752 (lactone), 1705 (cyclopentenone), 1660, 1595 and 1570 cm.⁻¹, but no hydroxyl band.

Anal. Calcd. for C₁₅H₁₈O₆: C, 73.75; H, 6.60. Found: 74.13; H, 6.93.

Tetrahydrohelenalin Mesylate.—Mesylation of 0.45 g. of tetrahydrohelenalin as described above gave 0.42 g. of crude product which was dissolved in 6 ml. of benzene and chromatographed over 5 g. of alumina. The first five benzene eluate fractions yielded 0.38 g. of product melting near 120°, subsequent fractions gave 0.08 g. of tetrahydrohelenalin and unidentified oils. Recrystallization from benzene-petroleum ether raised the m.p. to 126–127°.

Anal. Calcd. for C₁₆H₂₄O₆S: C, 55.77; H, 7.02; S, 9.30. Found: C, 55.33; H, 6.96; S, 9.30.

A mixture of 0.37 g. of the mesylate and 5 ml. of 2,6-lutidine was refluxed for 24 hr., cooled, poured into ice-hydrochloric acid and extracted with chloroform. The usual work-up resulted in 0.27 g. of partially-crystalline material which was dissolved in 6 ml. of benzene and chromatographed over 6 g. of alumina. Benzene and benzene-ether eluted 0.14 g. of V, m.p. 137° after recrystallization from benzene-petroleum ether, (α)²⁵D 73° (95% ethanol, C, 0.58), λ_{\max} 218, 291 m μ , ϵ_{\max} 15600 and 38, infrared bands at 1735 and 1670 (strong).

Anal. Calcd. for C₁₅H₂₀O₆: C, 72.56; H, 8.12. Found: C, 72.65; H, 8.10.

Extraction of *H. flexuosum*.—*H. flexuosum* Raf. was collected along the banks of the Ocklockonee River near U. S. Highway 90 West of Tallahassee in late summer 1956 (this is the location from which the first collection reported in ref. 3 was made), in Huron, Indiana, on U. S. Highway 50 in

late summer, 1956 and 1957 and on the Cumberland Plateau, Tennessee, along U. S. Highway 70 between Crossville and Crab Orchard, West of Daddy's Creek, in late August, 1958. Results of small-scale extractions from each of the collections gave flexuosin A in 0.38% (mainly leaves), 0.09% and 0.41% (mainly leaves) yield. Flexuosin B was obtained in yields of 0.24% (mainly leaves), 0.06% and 0.20% (mainly leaves). The experience thus gained permitted the development of the following procedure. The dried whole plant, wt. 33 lb., was crushed and extracted in a large Lloyd extractor with 60 l. of chloroform for two days. The extract was worked up in the usual way.³ The crude product, wt. 200 g., was taken up in 150 ml. of benzene and allowed to stand. Crystals of flexuosin A separated slowly. After two months, 26.5 g. of flexuosin A was removed by filtration and washed with a little benzene. The combined filtrate and washings were chromatographed over 700 g. of alumina, flexuosin B being eluted with benzene. The benzene eluates (six 230 ml. portions) were evaporated to dryness *in vacuo*; on crystallization of the residue from 35% ethanol was obtained 13.5 g. (0.09%) of flexuosin B. Benzene-chloroform (10:1 and 5:1) eluted an oil which could not be induced to crystallize. Benzene-chloroform (1:1), 1.7 l. of chloroform and 900 ml. of chloroform-methanol (20:1) eluted an additional 6.2 g. of flexuosin A after crystallization from benzene, total yield 32.7 g. (0.2%). Additional crystallizations from benzene, ethanol and ethyl acetate gave the analytical sample, m.p. 220–221.5°, mixed m.p. with neohelenalin from earlier³ extractions 198–207°, (α)²⁵D 12.4° (CHCl₃, C 4.93), infrared bands (CHCl₃) at 3400 (hydroxyl), 1755 (γ -lactone) 1725 and 1250 (in acetonitrile, acetate) and 1660 cm.⁻¹ (rel. strong, shoulder at 1640-double bond). The infrared spectrum was quite different from that of neohelenalin.

Anal. Calcd. for C₁₇H₂₄O₆: C, 62.95; H, 7.46. Found: C, 62.75; H, 7.48; C-methyl, 1.83 moles.

Flexuosin B was recrystallized from dilute ethanol, m.p. 132–137° (dec.), (α)²⁵D 44.2° (CHCl₃, C 4.78). The infrared spectrum had bands at 3400 (weak, OH?), 1760 (γ -lactone), 1740 and 1230 (acetate), 1710 (ketone), 1645 (strong, conjugated double bond) and 1600 (weak, double bond?).

Anal. Calcd. for C₁₇H₂₄O₆: C, 62.95; H, 7.46. Found: C, 62.80; H, 7.80; C-methyl, 1.88 moles.

Extraction of *H. campestre*.—*Helenium campestre* Small was collected in late May and June, 1959, near Greenbrier, Mt. Vernon, Morgantown and Clinton, Arkansas. Two chloroform extractions were carried out in the usual manner, the first utilizing 760 g. of flowers and small stems, the second 2000 g. of crushed whole plant. The crude extract from run 1 was dissolved in 200 ml. of hot ethanol and treated with a solution of 10 g. of lead acetate in 200 ml. of hot water containing a few ml. of acetic acid. After one day the orange solution was filtered, concentrated *in vacuo* and extracted with chloroform. Removal of chloroform gave 63 g. of orange-brown gum which refused to crystallize. In a typical experiment leading to the isolation of helenalin, 18 g. of the gum dissolved in 25 ml. of benzene, was chromatographed over 120 g. of alumina (Alcoa Grade F-20). A number of colored bands developed. Band 1 (green) on elution with benzene yielded 1.98 g. of gum, band 2 (yellow) gave 3.3 g. of gum, bands 3–5 (orange-yellow) gave 1.6 g. of gum. Benzene containing increasing proportions of ether yielded 0.8 g. of pale yellow product (fraction 6) which solidified on stirring. Benzene-ethanol mixtures eluted 3.4 g. of viscous material (fraction 7). Recrystallization of part of fraction 6 from benzene-petroleum ether gave helenalin, m.p. 165–166°, identical in all respects with authentic material. Rechromatography of the remainder of fraction 6 over alumina gave helenalin in the benzene-ether eluates, the total yield of crystalline helenalin amounting to 0.35 g. Rechromatography of fractions 1–5 and 7 failed to give crystalline material. The infrared spectra of the various gums indicated the presence of additional helenalin. Attempts to obtain crystalline fractions from run 2 failed.

TALLAHASSEE, FLORIDA