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CONSTITUENTS OF *HELENIUM* SPECIES—XVII

SESQUITERPENE LACTONES OF *HELENIUM THURBERI* GRAY AND THE STEREOCHEMISTRY OF BIGELOVIN[1,2]

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Abstract—Tenulin (I) and thurberilin, a new sesquiterpene lactone, were isolated from *Helenium Thurberi* Gray. The structure of thurberilin was shown to be II. Conversion of thurberilin to tetrahydrobigelovin established the structure and stereochemistry of bigelovin as XI.

SINCE our last summary[3] of work on constituents of *Helenium* species, new compounds have been isolated from *H. Bigelovii* Gray[4] (bigelovin) and *H. aromaticum* (Hook) Bailey[5] (aromatin, aromaticin) as well as from reinvestigations of *H. amarum* (Raf.) H. Rock[6] (amarilin, aromaticin) and *H. autumnale* L[7]. (dihydromexicanin E). Gross structures have been deduced for helenalin,[8] mexicanin A,[8] mexicanin C,[9] mexicanin E,[10] mexicanin I,[11] flexuosin A and flexuosin B.[1] Lastly, X-ray analyses of bromoisotenulin[12] and bromohelenalin,[13] in conjunction with the interpretation[9] of numerous correlations recorded in the literature permitted the assignment of absolute stereochemistry to many of these and other previously reported pseudoguaianolides.

In the present paper we wish to report our work on the constituents of yet another *Helenium* species, *H. Thurberi* Gray, whose distribution in the United States is limited to Southern Arizona. Extraction with chloroform and chromatography over alumina furnished tenulin (*I*, approx. 1% yield) and a new substance (0·1% yield) of formula $C_{20}H_{28}O_5$ which we have named thurberilin.

The UV spectrum of thurberilin, λ_{max} 223 and 320 mμ (ε 15500 and 54) suggested the presence of an α,β-unsaturated ketone function. The IR spectrum had bands at 1765, 1710 (double intensity), 1645 and 1595 cm^{-1}. The absorption at 1765 cm^{-1}

[1] Previous paper, W. Herz, Y. Kishida and M. V. Lakshmikantham, *Tetrahedron* **20**, 979 (1964). In this paper, the sense of a Note Added in Proof is garbled and is given correctly in *Tetrahedron* **20**, 1986 (1964).

[2] Supported in part by grants from the United States Public Health Service (GM-05814) and the National Science Foundation (GP-1492).

[3] W. Herz, *J. Org. Chem.* **27**, 4043 (1962).

[4] B. A. Parker and T. A. Geissman, *J. Org. Chem.* **27**, 4127 (1962).

[5] J. Romo, P. Joseph-Nathan and F. Diaz A., *Tetrahedron* **20**, 79 (1964).

[6] R. A. Lucas, S. Rovinski, R. J. Kiesel, L. Dorfman and H. B. MacPhillamy, *J. Org. Chem.* **29**, 1549 (1964).

[7] R. A. Lucas, R. G. Smith and L. Dorfman, *J. Org. Chem.* **29**, 2101 (1964).

[8] W. Herz, A. Romo de Vivar, J. Romo and N. Viswanathan, *J. Amer. Chem. Soc.* **85**, 19 (1963).

[9] W. Herz, A. Romo de Vivar, J. Romo and N. Viswanathan, *Tetrahedron* **19**, 1359 (1963).

[10] J. Romo, A. Romo de Vivar and W. Herz, *Tetrahedron* **19**, 2317 (1963).

[11] E. Dominguez and J. Romo, *Tetrahedron* **19**, 1415 (1963).

[12] D. Rogers and Mazhar-ul-Haque, *Proc. Chem. Soc.* 92 (1963).

[13] M. T. Emerson, C. N. Caughlan and W. Herz, *Tetrahedron Letters* No. 12, 621 (1964).

immediately indicated that we were dealing with a γ-lactone and the combination of 1710 and 1595 cm^{-1} that thurberilin was an α,β-unsaturated cyclopentenone of the type found in tenulin,[14] helenalin[8] and many other pseudoguaianolides. The extra bands at 1710 and 1645 cm^{-1}, coupled with the molecular formula, suggested that thurberilin was an ester of an α,β-unsaturated 5-carbon acid.

The nature of this acid was revealed by ozonolysis, which liberated acetaldehyde, and by hydrolysis which furnished tiglic acid. Hence thurberilin was the tiglate or angelate of a sesquiterpene lactone, whose UV spectrum resulted from the superposition of this chromophore on that of the α,β-unsaturated cyclopentenone.

The NMR spectra of thurberilin and of its derivatives provided support for the provisional structure II (exclusive of stereochemistry) which was eventually confirmed by correlation with tenulin. Two low-field quartets at 7·57 (J = 6, 2) and 5·99 ppm (J = 6, 3)[15] thoroughly mirrored analogous signals characteristic of H_2 and H_3 in the NMR spectra of helenalin and tenulin and disappeared on catalytic reduction of thurberilin. Two additional low-field protons at 5·42 d (J = 8) and 4·61 td (12, 3) were ascribed to H_6 and H_8. Experience dictates that the signal at lower field is that of the ester hydrogen which because of its multiplicity must be attached to C_6. This conclusion was verified by the NMR spectrum of desangeloyldihydrothurberilin (VI) which retained the multiply-split signal of H_8 near 4·6 ppm but in which the H_6 doublet had moved, as expected, to higher field.

The usual methyl signals, one singlet at 1·28 and two methyl doublets at 1·29 and 1·19 ppm (J = 6) were also found but superimposed on these familiar features were observed the signals of the 5-carbon side chain, two vinyl methyl groups at 1·97 (doublet of quartets, splittings 6·5, 1·5) and 1·73 (apparent quintet, 1·5) and one vinyl proton at 5·99 ppm.[16,17] On catalytic hydrogenation to tetrahydrothurberilin (III), the vinyl methyl signals moved upfield, merging with the methyl signals of the sesquiterpene moiety, and the signals of the vinyl hydrogen disappeared. The chemical shift of the latter was characteristic of an angelate (approx. 6 ppm) rather than a tiglate (approx. 6·7 ppm),[17] as was the long-range splitting (1·5 c/s) of the vinyl methyl groups. Hence the side chain possesses the *trans* configuration of angelic acid.

Catalytic hydrogenation of thurberilin with platinum oxide furnished tetrahydrothurberilin (III). With Pd—C there was formed a mixture of III and dihydrothurberilin (IV) which was difficult to separate. However, when the mixture was subjected to ozonolysis, III was recovered while IV was cleaved to acetaldehyde and a pyruvate V whose lactone ring was closed to C_8 (NMR spectrum). Compound V was easily hydrolyzed to VI under conditions which have been shown not to involve lactone ring orientation (K_2CO_3—MeOH), while the hydrolysis of III to VI required somewhat stronger base.

Conversion of VI to the mesylate followed by treatment with lutidine resulted in isolation of a substance which was identical in all respects with anhydrodesacetyldihydroisotenulin (VII) of established structure[14] and stereochemistry.[9] Since VI

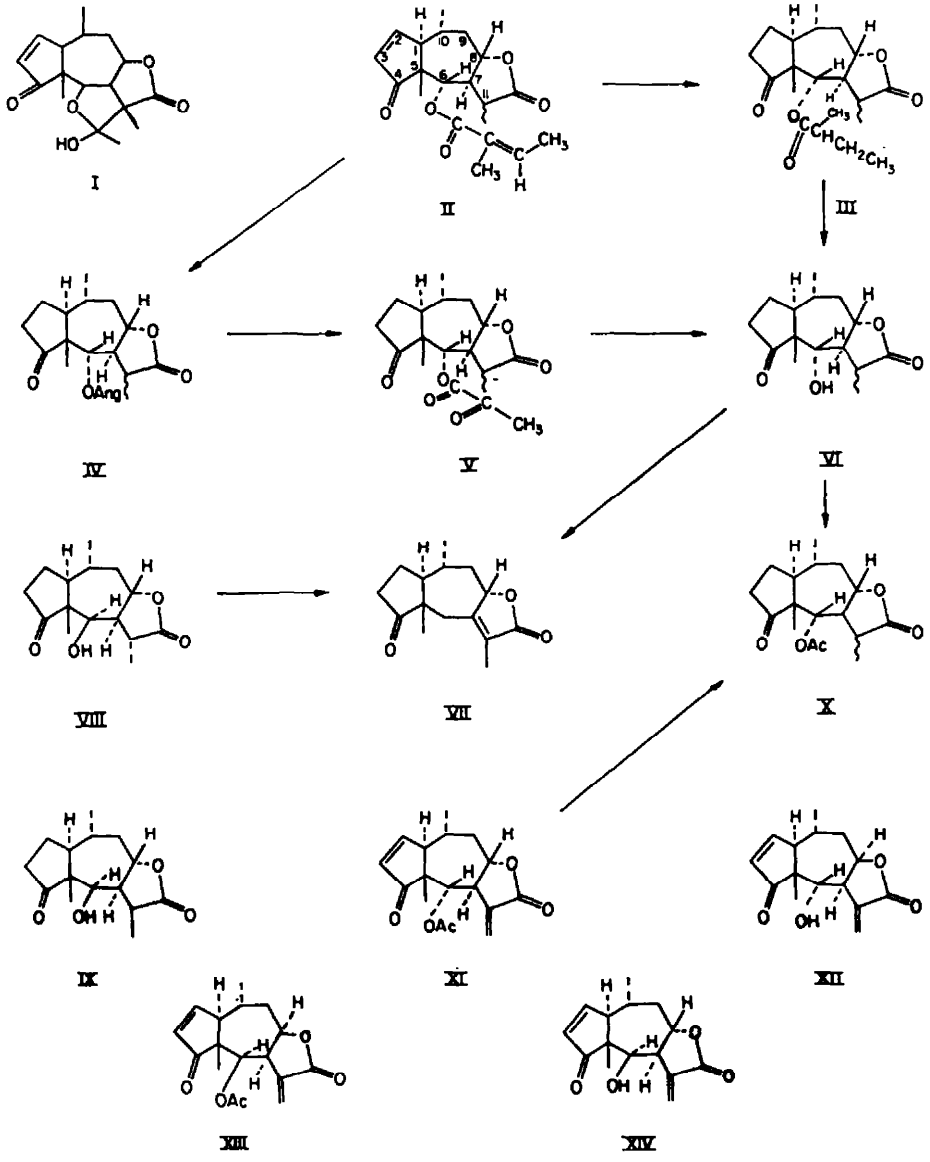
[14] W. Herz, W. A. Rohde, K. Rabindran, P. Jayaraman and N. Viswanathan, *J. Amer. Chem. Soc.* **84**, 3857 (1962).

[15] NMR spectra were run on an A-60 spectrometer in $CDCl_3$ with tetramethylsilane serving as internal standard. Symbols for multiplets are the ones previously used in this series of papers.

[16] This signal, theoretically a quartet of quartets,[17] was exactly superposed on that of H_3.

[17] R. R. Frazer, *Canad. J. Chem.* **38**, 549 (1960).

differs from desacetyldihydroisotenulin (VIII) and from its C_{11}-epimer tetrahydromexicanin I[11] (IX) it must be represented by the stereochemistry shown (H_6 β) where



the only uncertainty is the configuration of the C_{11}-methyl group. Thurberilin is therefore II.

Acetylation of VI furnished a substance X which was identical in all respects with an authentic sample of tetrahydrobigelovin.[4,18] Hence bigelovin must be XI and, contrary to earlier speculations,[19] possesses the same *trans*-bicyclo (5, 3, 0) structure (H,-α, C_5-methyl β) as the remaining three members of the quartet of compounds

[18] We are grateful to Professor T. A. Geissman for material which permitted the comparison.

epimeric at C_6 and/or C_8, i.e. helenalin (XII),[9] balduilin (XIII)[9] and mexicanin I (XIV).[11] The unusual order of hydrogenation exhibited by the two double bonds of bigelovin[4] must be due to the combination of configurations at C_6 and C_8, a circumstance which perhaps also accounts for the simultaneous formation of III and IV during the hydrogenation of thurberilin.

Tetrahydrobigelovin (X) is thus the seventh of the eight possible stereoisomers possessing the same stereochemistry at C_1, C_5, C_7 and C_{10}, but differing at C_6, C_8 and C_{11}. Its optical rotatory dispersion curve displays the usual strongly positive Cotton effect characteristic of *trans* (1α-) sesquiterpene lactones of this genre.[8,14,20] The eighth as yet unknown isomer, epimeric with X at C_{11}, is probably the less stable epimer since tetrahydrobigelovin is unaffected by treatment with potassium carbonate in xylene.[21]

EXPERIMENTAL[22]

Extraction of Helenium thurberi *Gray. H. Thurberi* Gray was collected by Mr. R. J. Barr at Tanque Verde Creek southwest of State Road 49, Tucson, Pima County, Arizona on July 14 and 15, 1963. (Barr No. 63-289). The ground whole plant, wt. 24 lbs, was extracted in the usual fashion.[1] The crude gum was dissolved in the minimum of benzene–$CHCl_3$ (2:5) and adsorbed on 3 kg alumina (Alcoa F-20) deposited with benzene. Several cuts of benzene and benzene–$CHCl_3$ failed to elute anything. The eluent was changed to $CHCl_3$ which eluted 7·3 g crude thurberilin first. The later fractions furnished tenulin, isotenulin, and desacetylneotenulin[23] as well as milligram quantities of other substances. These results essentially duplicated the yields from the initial collection (Barr 60–198) made at the same location on Aug. 12, 1960 (no isotenulin and desacetylneotenulin isolated) and from a collection made in the Santa Cruz River drainage area along Silverlake Road near Tucson on April 30, 1963 (Barr No. 63-167A). However, no thurberilin, only tenulin was obtained when several small collections made along the San Pedro River near Benson, Arizona and along Silverlake Road from May–Sept. 2, 1962 and along Tanque Verde Creek and the Santa Cruz River near Tucson from April–July 1961 were combined and extracted.

Thurberilin was recrystallized from acetone–isopropyl ether, m.p. 162°, $\nu_{cm^{-1}}$ 1765, 1710 (double intensity), 1645 and 1595; λ_{max} 223, 320 mμ (ε_{max} 15,500 and 54); $[\alpha]_D^{27°} = +20°$ ($c = 0{\cdot}4$). (Found: C, 68·83; H, 7·59; O, 23·84. Calcd. for $C_{19}H_{26}O_5$: C, 69·34; H, 7·57; O, 23·09%.)

Hydrolysis of thurberilin. Thurberilin, wt. 100 mg was treated with 15 ml 5% alcoholic KOH solution in the cold overnight. The solvent was then removed and the residue treated with ice and H_2SO_4 (pH 2). The acidified solution was steam-distilled; the steam-volatile acid, wt. 15 mg, was isolated from the distillate by ether extraction and identified as tiglic acid (m.p. 60°) by m.p. and m. m.p. determination. Also the IR spectra were superimposable.

Ozonolysis of thurberilin. A solution of 0·1 g thurberilin in $CHCl_3$ was ozonized at −30°. The $CHCl_3$ was removed at low temp *in vacuo* and the residue steam distilled into a solution of 2,4-dinitrophenylhydrazine hydrochloride. The solid which separated was recrystallized repeatedly, m.p. 155°, and was identified as acetaldehyde 2,4-dinitrophenylhydrazone by mixed m.p. determination, TLC and comparison of IR spectra.

[19] J. B. Hendrickson, *Tetrahedron* **19**, 1387 (1963).

[20] C. Djerassi, J. Osiecki and W. Herz, *J. Org. Chem.* **22**, 1361 (1957).

[21] Compound VI is a glass and differs from "desacetyltetrahydrobigelovin", m.p. 221–223°, obtained in small amount by treatment of X with sodium methoxide in absolute methanol.[4] It is possible that the formation of this substance, whose NMR spectrum is unfortunately unrecorded, was accompanied by lactone ring reorientation and that it possesses the gross structure of desacetyldihydroalloisotenulin.[14]

[22] M.ps are uncorrected. Analyses were by Dr. F. Pascher, Bonn, Germany. UV spectra and rotations were determined in 95% EtOH; IR spectra in $CHCl_3$ unless otherwise specified.

[23] It is not clear whether isotenulin and desacetylneotenulin are artefacts formed during the long exposure of tenulin to the basic alumina necessitated by the tedious chromatogram of large batches. Attempts to establish this by thin-layer chromatography of the crude extract failed due to the number of overlapping spots which could not be resolved.

Tetrahydrothurberilin (III). A solution of 0·4 g thurberilin in 50 ml ethyl acetate was reduced at 37 lbs press. in the presence of 0·1 g PtO_2. After 4 hr, the solution was filtered. The residue obtained after evaporation of the solvent was recrystallized from acetone–isopropyl ether to give 0·15 g tetrahydrothurberilin, m.p. 185°. The mother liquors on evaporation furnished more III (TLC, IR spectrum) which had m.p. 165°. The product had IR bands at 1775 and 1750 (double intensity) cm^{-1}, $[\alpha]_D^{27}$ (*c*, 0·0805) +114·3°, ORD curve (*c*, 0·0925, MeOH), $[\alpha]_{600}$ −118°, $[\alpha]_{589}$ +125°, $[\alpha]_{320}$ +1622°, $[\alpha]_{312-15}$ +1510°, $[\alpha]_{280}$ −804°, $[\alpha]_{250}$ +277° (last reading); a + 78·5°, NMR signals at 4·50 td (10, 3, H_a) and 5·30 d (8, H_a). Signals in the methyl region were complex and could not be disentangled, total intensity 15 protons. (Found: C, 68·45; H, 8·46. Calcd. for $C_{20}H_{30}O_5$: C, 68·57; H, 8·57%.)

Reduction of thurberilin at atm. press, in ethyl acetate solution in the presence of Pd—$CaCO_3$ catalyst gave a mixture of IV and III as evidenced by TLC which showed two spots, one corresponding to III, IR spectrum (bands at 1775, 1750, 1723 and 1650 cm^{-1}), and the NMR spectrum which had signals corresponding to the vinyl proton and the vinyl methyl groups of the angeloyl side chain superimposed on the spectrum of III. Reduction of the crude mixture at 37 p.s.i. in the Parr hydrogenator, using PtO_2 as catalyst, furnished a quantitative yield of III.

Ozonolysis of the crude di- and tetrahydrothurberilin mixture, wt, 0·5 g, in $CHCl_3$ solution at −30° in the manner described above, led, after steam distillation into a solution of 2,4-dinitrophenylhydrazine hydrochloride, to material, m.p. 155°, identified as acetaldehyde 2,4-dinitrophenylhydrazone by m. m.p. determination, TLC and comparison of the IR spectra.

The non-volatile component was extracted with $CHCl_3$ and the organic layer washed, dried and evaporated. The residues from two ozonolyses, total wt. 0·66 g, were combined and chromatographed over acid-washed alumina to give 0·36 g tetrahydrothurberilin, originally present in the hydrogenation mixture, and 0·207 g pyruvate V, m.p. 164° after recrystallization from acetone–pet. ether, $[\alpha]_D^{25}$ +120° (*c* 0·55), IR bands at 1779 and 1739 (high intensity) cm^{-1}, NMR signals at 1·09 d (6) and 1·15 d (7, C_{10}- and C_{11}-methyl), 1·12 (C_5-methyl), 2·40 (acetyl), 4·47 tc (11, H_a) and 5·33 d ppm (8, H_a). (Found: C. 64·11, H, 7·18, O, 28·83. Calc. for $C_{18}H_{24}O_6$: C, 64·27; H, 7·19; O, 28·54%.)

Hydrolysis of V. A solution of 0·15 g V in 15 ml EtOH was refluxed for 1 hr with 0·3 g K_2CO_3. The solvent was removed at red. press., ice and dil HCl aq were added and the whole extracted with $CHCl_3$. The organic layer was washed, dried and evaporated. The glassy residue (VI) could not be crystallized even on extensive chromatography, but was homogeneous (TLC), IR bands at 3700, 1775 and 1740 cm^{-1}.

Tetrahydrobigelovin (X). A solution of 0·6 g VI in 2 ml dry pyridine was allowed to stand with 1 ml acetic anhydride at room temp overnight. The acetyl derivative, wt. 0·34 g, was isolated as usual and when crystallized from acetone–pet. ether melted at 205°, $[\alpha]_D^{27}$ +118° (*c*, 0·0492), $[\alpha]_D^{27}$ +145·4° (*c*, 0·180, $CHCl_3$), IR bands at 1775 and 1750 (double intensity) cm^{-1}, NMR signals at 0·92 d and 1·15 d (J = 7, C_{10}- and C_{11}-methyls), 1·08 (C_5-methyl), 4·46 (C_{18}, H_a) and 5·25 d (J = 8, H_a), ORD curve (*c*, 0·013, MeOH) $[\alpha]_{600}$ +246°, $[\alpha]_{589}$ +246°, $[\alpha]_{318}$ +2030°, $[\alpha]_{279}$ −985°, $[\alpha]_{250}$ −339° (last reading), a = +47. The m. m.p. with authentic tetrahydrobigelovin[18] was not lowered. Both samples showed the same R_f values on TLC and had identical IR spectra. Parker and Geissman, however, report $[\alpha]_D$ +187° ($CHCl_3$). (Found: C, 65·85; H, 7·64. Calc. for $C_{17}H_{24}O_5$: C, 66·21, H, 7·85%.)

Anhydrodeacetyldihydroisotenulin (VII). Hydrolysis of 0·1 g III with ethanolic KOH (1·5 g in 75 ml) at room temp also furnished a non-acidic component which could not be crystallized and was identified as VI by TLC, IR spectrum and acetylation to tetrahydrobigelovin. Crude VI, wt. 0·3 g, was dissolved in 3 ml dry pyridine, mixed with 1·5 g methanesulphonyl chloride and left overnight. The gummy mesylate was isolated as usual and refluxed with 4 ml 2,6-lutidine for 8 hr. The dark solution was cooled and poured into ice and HCl aq. The neutral fraction was isolated in the usual manner and weighed 0·212 g. Chromatography over acid-washed alumina furnished 0·086 g VII, m.p. 142°, IR bands at 1750 and 1650 cm^{-1}, $[\alpha]_D^{25}$ +175° (*c*, 0·3225), m.p. undepressed on admixture of authentic material, IR spectra superimposable.

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