

[CONTRIBUTION FROM THE BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, UNITED STATES DEPARTMENT OF AGRICULTURE]

The Constituents of Certain Species of *Helenium*. II. Tenulin

BY E. P. CLARK

In the first paper of this series¹ attention was directed to a statement by Reeb² that helenalin from *Helenium autumnale* is a vermifuge. The writer has found it to be a fairly effective fish poison, and in unpublished experiments entomologists of this Bureau have shown that it has possibilities as an insecticide. These facts stimulated investigation of other species of the genus for possible sources of helenalin or other materials likely to be related to it. The results of this work have shown that *Helenium macrocephalum* also contains helenalin, while three other species—*H. tenuifolium*, *H. elegans*, and *H. badium*—contain a crystalline substance hitherto unreported. This material has been named "tenulin" from *H. tenuifolium*, the plant from which it was first obtained.

Tenulin was prepared from the species of *Helenium* mentioned above by extraction and crystallization from organic solvents. Because of the retention of solvent of crystallization, it separates from a number of such solvents with different melting points, but after undergoing some purification it may be recrystallized from water or aqueous organic solvents and then has a constant composition represented by $C_{17}H_{22}O_5$ and a melting point of 193–195°.

It is bitter and mildly sternutative and has a pronounced toxic effect upon fish in a concentration of 1–5000.

Tenulin is optically active; $[\alpha]^{20}_D$ is -21.7° in ethanol. Reactions for hydroxyl, carboxyl, and carbonyl groups have so far given negative results. Alkoxy groups are absent, but it does give a small quantity of alkyl iodide, which when calculated to methoxyl corresponds to approximately 0.5%. Hydrogenation of tenulin yields a dihydro compound which readily gives a phenylhydrazone, whereas the unreduced material does not. Upon bromination, two atoms of bromine are added, indicating a double bond or its equivalent. One of the bromine atoms, however, is readily lost, and a monobromo compound is formed. When a small tube containing tenulin is plunged into a bath at 300°, the material melts with the rapid

evolution of gas. As the bath cools to 275°, the reaction is complete and a golden-colored melt results, which yields a crystalline substance, m. p. 172°. This has been shown by analysis to be anhydrotenulin, $C_{17}H_{20}O_4$. The action of acetic anhydride and sodium acetate upon tenulin is anomalous. A product is obtained which analyzes for $C_{22}H_{26}O_5$ but does not contain acetyl.

Mild treatment of tenulin with alkali converts it to an isomer which melts, according to the method of making it, at 158–162°. The following procedures were used to obtain this isomer, which has been designated isotenulin: (1) triturating finely powdered tenulin with 0.1 *N* sodium hydroxide for three minutes; (2) triturating finely powdered tenulin with 5% sodium carbonate solution and allowing the mixture to stand for periods of fifteen minutes to twenty-four hours; (3) boiling an aqueous solution of tenulin with norit, which because of the alkaline reaction imparted to the solution by the carbon caused isomerization; and finally (4) treating an aqueous methanolic solution with a small quantity of 1% sodium hydroxide.

According to the procedure used, practically pure isotenulin or a mixture of isotenulin and a new substance, $C_{15}H_{20}O_4$, m. p. 255°, may be obtained.

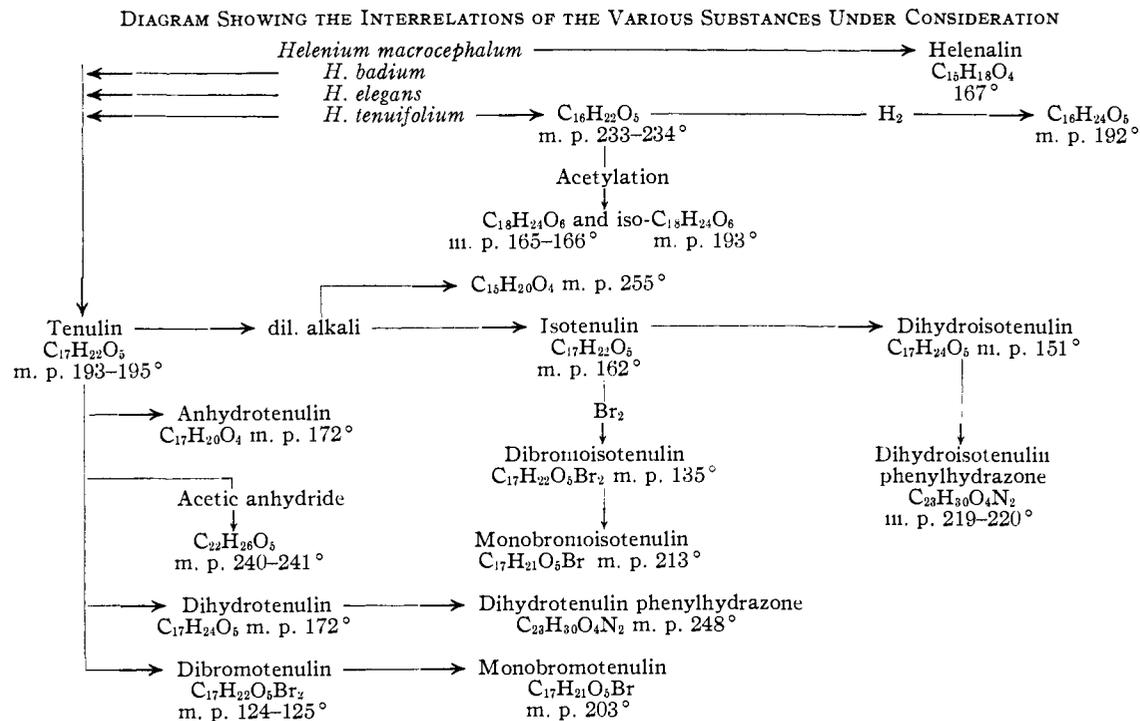
Isotenulin is of interest because it is the compound described in 1937 by Buehler, Whitehead, and Goodge,³ which they obtained from *Helenium tenuifolium*. These investigators reported upon an indefinite material which melted over a range of 152–170°. It was not purified or analyzed, but upon treatment with dilute sodium carbonate solution gave a well-defined compound, $C_{17}H_{22}O_5$, which melted at 158°. This is the material designated above as isotenulin. They record three derivatives of it: a monobromo compound, m. p. 200°; a dihydro compound, m. p. 150.8°; and a phenylhydrazone of the latter, m. p. 209–210°.

A comparison of the melting points recorded by Buehler and co-workers for these substances with isotenulin and the corresponding derivatives which were made for the purpose agree only in two

(1) Clark, *THIS JOURNAL*, **58**, 1982 (1936).

(2) Reeb, *J. Pharm. Elsas Lothringen*, **37**, 149 (1910).

(3) Buehler, Whitehead and Goodge, *THIS JOURNAL*, **59**, 2299 (1937).



instances, namely, isotenulin and its dihydro derivative. The phenylhydrazone of dihydroisotenulin melts at 219–220°, and bromination of isotenulin according to Buehler's method yields a dibromo compound, m. p. 135°. This, however, can be converted readily to a monobromo derivative, m. p. 213°. In spite of these differences, it is quite safe to say that the material obtained by Buehler and co-workers is isotenulin, for in working up plant extracts according to their directions the writer obtained isotenulin.

The preparation of tenulin from different lots of *Helenium tenuifolium* was accompanied by considerable variation in yields and in the ease with which the material was obtained. Some samples contained relatively large quantities of non-crystallizable material, which greatly inhibited the crystallization of tenulin. One shipment of *H. tenuifolium*, obtained in 1936, gave with difficulty poor yields of crystalline material, while the same drug, obtained in 1934 from essentially the same location, gave a clean extractive from which tenulin was obtained with ease.

Upon treatment for purification the material used in 1936 was found to consist of about 60% of tenulin and 40% of another compound, $C_{16}H_{22}O_5$. This C_{16} material gave a dihydro derivative, $C_{16}H_{24}O_5$, and two isomeric monoacetyl derivatives. One of these, obtained by the action of

boiling acetic anhydride and sodium acetate, melted at 165–166°; the other, made by allowing the material to react with acetic anhydride and cold pyridine, melted at 193°. The new compound contains no alkoxy groups, but under the conditions employed to determine these groups an alkyl iodide was obtained, which when calculated to methoxyl corresponded to 3.8%. This reaction strongly indicates a diortho-substituted isopropyl group.⁴

No attempt is made here to interpret the reactions under consideration, but they serve to characterize tenulin, show its relation to Buehler, Whitehead, and Godge's compound, and indicate the occurrence of several apparently closely related compounds in certain species of *Helenium*. A summary of the sources of the various products under consideration and their relation to each other is given in the accompanying diagram.

Experimental

Helenalin from *Helenium macrocephalum*.—This plant, which yields helenalin, was treated in the manner described for obtaining helenalin from *H. autumnale*.¹

Preparation of Tenulin from Different Plant Sources.—The following procedure for preparing tenulin is the most satisfactory of several employed. This method was used in processing all three species of *Helenium* containing tenulin mentioned before.

(4) Clark, *THIS JOURNAL*, **51**, 1479 (1929); Adams and Butterbaugh, *ibid.*, **60**, 2174 (1938).

Twenty-five hundred grams of ground whole plant was moistened with 2 liters of chloroform and allowed to stand in a tight container overnight. The drug was then packed in a percolator and extracted with 10 liters of chloroform. The extract was concentrated under diminished pressure to a thick sirup and then dissolved in 1.5 liters of hot ethanol. The alcoholic liquid was diluted with 6 liters of boiling water and acidified (litmus) with acetic acid, and a solution of 20 g. of normal lead acetate was added. After standing overnight the solution cleared and was filtered through a folded filter paper. The filtrate was evaporated under reduced pressure to 1 liter and then extracted with chloroform. The chloroform extract was dried with sodium sulfate and evaporated under reduced pressure to a thick sirup, which was dissolved in boiling benzene and allowed to crystallize.

This procedure gave satisfactory results with all species of *Helonium* except occasionally *tenuifolium*. In these cases the thick chloroform extract was dissolved in 250 cc. of methanol, 75 cc. of ethylene glycol was added, and most of the methanol removed by distillation under reduced pressure. The resulting glycol solution was removed from the flask with the aid of 20 cc. of hot methanol and treated with water until turbidity occurred. It was then placed in a cool box and allowed to crystallize. The yield was usually 1.5% of the weight of the drug.

The purification of the material obtained by either of the foregoing procedures was accomplished in several ways:

(1) Five grams of crude tenulin was dissolved in 20 cc. of hot ethylene glycol. To this solution was added 60 cc. of water. Crystallization soon began and yielded 3.6 g., m. p. 183°.

(2) Tenulin was dissolved in the necessary quantity of *n*-butanol, and 4 volumes of *n*-butyl ether was added. The material melted at 191° and cleared at 196°. The melting point frequently varied, however, as illustrated in the following experiment. Tenulin with a melting point of 191° was recrystallized several times from butanol and butyl ether as above. The first recrystallization gave a product which melted at 178°, the second at 183°, the third at 186°, and the fourth at 175°. In all cases the product separated as dense prisms. Apparently all the different specimens were pure but contained solvent.

(3) The best method thus far found was to dissolve the crude substance in acetone and add to this a relatively large quantity of hot water (250 cc. of water to 5 g. of tenulin), then evaporate the resulting liquid under reduced pressure, and remove the crystals from time to time. When pure material thus prepared was allowed to dry in the air, it melted at 193–195° and analyzed for a compound with the composition $C_{17}H_{22}O_5$.

(4) Highly colored material was purified as follows. A solution of 10 g. of substance in 10 cc. of boiling *n*-butanol was diluted with 80 cc. of benzene and allowed to crystallize. This removed much of the color, and it could then be recrystallized from water.

When pure and recrystallized from water, tenulin consists of six-sided plates, frequently forming clusters which break into angular fragments. It melts at 193–195°. Its indices of refraction, determined by the immersion method, are: n_α , 1.525; n_β , 1.565; n_γ , 1.585; all ± 0.003 . All the indices are readily found. The birefringence is extremely

strong, and the polarization colors are brilliant. In convergent polarized light (crossed nicols) partial biaxial figures are seen on many of the plates. The optic axis is perpendicular to many of them. The acute bisectrix was difficult to obtain; therefore, a determination of the axial angle could not be made.⁵

Tenulin is optically active; 115.46 mg. dissolved in 2.28 cc. of ethanol ($C = 5.2$) at 20° rotated the plane of polarized light in a 96-mm. tube 1.1° to the left; $[\alpha]^{20}_D$ is -21.6° . 4.74 mg. of tenulin dissolved in 49.95 mg. of camphor; Δ , 11.75°. This corresponds to a molecular weight of 323.

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.65; H, 7.27; mol. wt., 306.4. Found: C, 66.52; H, 7.51; mol. wt., 323. Recrystallized from *n*-butanol and *n*-butyl ether, m. p. 191. Found: C, 66.61; H, 7.47.

Dihydratenulin.—One gram of tenulin dissolved in 50 cc. of ethyl acetate was reduced at atmospheric pressure with hydrogen and the platinum catalyst of Voorhees and Adams.⁶ One mole of hydrogen was absorbed. The catalyst was removed and the solution evaporated to 5 cc. To this 10 cc. of benzene was added. There was obtained 0.9 g. of colorless crystals, which sintered at about 160°, with the evolution of benzene (benzene of crystallization). The material finally melted at 180°.

This was recrystallized from its solution in 10 cc. of methanol upon the addition of three volumes of hot water. Thus obtained, it consisted of short, thick rods, which melted at 182°. The rods appeared to contain solvent of crystallization, for upon exposure to the air for some time the crystals became broken and had an effloresced appearance. It then melted at 172°. The material in this condition was analyzed.

Anal. Calcd. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84. Found: C, 66.05; H, 7.83.

Dihydratenulin Phenylhydrazone.—An ethanolic solution of 0.1 g. of dihydratenulin, 0.1 g. of phenylhydrazine, and 2 drops of acetic acid was diluted with sufficient water to produce cloudiness. A drop of ethanol was then added to clarify the liquid. Upon standing for a few minutes colorless rods, m. p. 238–240°, separated. These were recrystallized from their methanolic solution upon the addition of water. The purified material consisted of long, thin, square-ended rods, which melted and flowed with decomposition at 248°.

Anal. Calcd. for $C_{23}H_{30}O_4N_2$: N, 7.03. Found: N, 6.96.

Dibromotenulin.—An ethyl acetate solution of 0.5 g. of tenulin was treated portionwise with 0.26 g. of bromine (2 atoms) dissolved in ethyl acetate. The reaction was rapid, and when it was complete the ethyl acetate was removed by distillation under reduced pressure. The residue was dissolved from the flask with 10 cc. of methanol and poured into 200 cc. of water. The white amorphous precipitate was removed and air-dried. The yield was 0.75 g. The reaction was entirely addition, as no hydrobromic acid was formed.

(5) The optical data recorded in this communication were determined by George L. Keenan, of the Food and Drug Administration of the U. S. Department of Agriculture.

(6) Voorhees and Adams, *THIS JOURNAL*, **44**, 1397 (1922).

The material was crystallized from its solution in the necessary quantity of boiling methanol by adding 0.5 volume of hot water. It separated as rosetts of fine colorless needles and melted with decomposition at 124–125°. Further recrystallization did not change its melting point.

Anal. Calcd. for $C_{17}H_{22}O_5Br_2$: Br, 34.3. Found: Br, 34.1.

Monobromotenulin.—After standing for two to three days, the mother liquors from recrystallizing dibromotenulin became acid to congo red and deposited a white material. This was separated and dissolved in hot methanol. The solution was filtered and then crystallized by adding an equal volume of hot water. Thin, colorless, rectangular plates, melting at 198–199°, were obtained. When recrystallized from dilute methanol, it melted with decomposition at 202–203°.

Anal. Calcd. for $C_{17}H_{21}O_5Br$: Br, 20.7. Found: Br, 20.3.

Anhydrotenulin.—A test-tube containing 0.5 g. of tenulin was placed in a small bath of fused sodium and potassium nitrates at 300°. The tenulin melted with the evolution of gas. By the time the bath had cooled to 275° the reaction was complete, and a golden-colored melt resulted. This was dissolved in 2 cc. of boiling methanol, and an equal volume of water was added. Crystallization began at once and yielded 0.35 g. of crystals which melted at 163°. These were dissolved in methanol, and the filtered solution was crystallized by adding an equal volume of water. The product consisted of colorless crystals which melted at 172°.

Anal. Calcd. for $C_{17}H_{20}O_4$: C, 70.81; H, 6.99; mol. wt., 288.3. Found: C, 71.03; H, 7.09; mol. wt., 294.

Action of Acetic Anhydride and Sodium Acetate upon Tenulin.—A mixture of 2 g. of tenulin, 15 cc. of acetic anhydride, and 0.5 g. of dry sodium acetate was boiled for one hour. Most of the excess anhydride was removed by distillation, and the residue was treated with water. After standing for some time, with occasional stirring, the resulting oil was dissolved in methanol. Crystallization began at once. There was obtained 0.6 g. of material which melted at 230°. It was recrystallized from boiling butanol and when pure melted at 240°.

Anal. Calcd. for $C_{22}H_{26}O_5$: C, 71.33; H, 7.08; mol. wt. 370.4; $C_{22}H_{28}O_5$: C, 70.94; H, 7.58; mol. wt. 372.4. Found: C, 71.21, 71.18; H, 7.27, 7.18; mol. wt. (Rast), 355; acetyl was negative.

Isotenulin.—One gram of finely powdered tenulin in a mortar was triturated for three minutes with 50 cc. of 0.1 *N* sodium hydroxide. The mixture was then acidified with sulfuric acid, and a crystalline material with a melting point of 158° was obtained. The yield was 0.75 g.

It was recrystallized from its solution in the necessary quantity of *n*-butanol by adding four volumes of *n*-butyl ether. The product consisted of long colorless rods, which melted at 162°. Its indices of refraction are: η_α 1.519; η_β not determined, only faint interference figures were observed: η_γ 1.555, both ± 0.003 . η_α is shown lengthwise, and η_γ is shown crosswise on the rods. In parallel polarized light (crossed nicols) the polarization colors are brilliant, the extinction is parallel, and the elongation is negative. In convergent polarized light (crossed nicols) only

faint biaxial figures showing the acute bisectrix are observed. Apparently 2E is large.

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.81; H, 7.37.

When finely powdered tenulin was triturated for fifteen minutes with 5% sodium carbonate solution and then filtered, almost pure isotenulin, m. p. 162°, was obtained.

A solution of 0.5 g. of tenulin dissolved in 80 cc. of water was boiled for two minutes with 0.5 g. of norit. Upon cooling, isotenulin separated as colorless needles, m. p. 160–161°. The identity of the material was established by comparing it with an authentic sample made by the action of sodium carbonate upon tenulin. There was no depression of the melting point. Since norit suspended in water imparts to the latter an alkaline reaction, *pH* 7.5, it was assumed that the alkali was the cause of the transformation. This was proved to be the case, since an aqueous solution of tenulin acidified with acetic acid and then treated with norit failed to isomerize.

Still another procedure which yielded isotenulin is the following. To a solution of 0.5 g. of tenulin dissolved in 2 cc. of boiling methanol was added 10 cc. of hot water. Immediately 1 cc. of 1% sodium hydroxide solution was added. The turbid liquid was stirred for three minutes, during which time crystallization occurred. It was then acidified and allowed to stand for a few minutes, and the crystals, m. p. 157°, were removed. The yield was 0.37 g.

The Substance, m. p. 255°, Obtained by the Action of Alkali upon Tenulin.—When finely powdered tenulin is triturated occasionally during twenty-four hours with a 5% sodium carbonate solution, isotenulin is the main product of the reaction, but another substance also is formed. One gram of tenulin thus treated yielded 0.7 g. of yellow crystals, which melted unsharply at 155°. These were suspended in 5 cc. of hot methanol, and the mixture was then cooled and filtered. There was obtained 0.1 g. of a substance which melted at 241° and cleared at 246°. This was recrystallized to constant melting point from *n*-butanol. It consisted of short, thick, colorless rhombs, which melted at 255°. Analysis showed that the material had the composition $C_{15}H_{20}O_4$.

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.08; H, 7.69.

Upon dilution with three volumes of water, the methanolic mother liquors from which the foregoing substance was obtained gave light yellow isotenulin, m. p. 160°.

The material that melted at 255° is also formed when finely powdered tenulin is triturated occasionally with 0.1 *N* sodium hydroxide for one and one-half hours. The yield is essentially the same as that obtained with sodium carbonate, but no isotenulin could be obtained by this procedure.

Dihydroisotenulin.—This compound was prepared in the same manner as dihydrotenulin. The ethyl acetate solution was evaporated to dryness, dissolved in methanol, and crystallized by adding water. The crude material had a melting point of 150°. It was recrystallized from its methanolic solution by adding two volumes of boiling water. It separated as thick, colorless prisms, many of which were rhombs. Its melting point was 151°.

Anal. Calcd. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84. Found: C, 66.30; H, 8.07.

Dihydroisotenulin Phenylhydrazone.—This material was prepared by the same procedure as dihydrotenulin phenylhydrazone. When recrystallized by adding water to its methanolic solution, it melted at 219–220°.

Anal. Calcd. for $C_{28}H_{30}O_4N_2$: N, 7.03. Found: N, 7.00.

Dibromoisotenulin.—This compound was made in the same manner as dibromotenulin, and also by the procedure given by Buehler and co-workers.³ In each case the material was purified by dissolving it in the necessary quantity of boiling methanol and adding 0.5 volume of hot water. It separated as dense, irregular-shaped prisms tending to be octahedra. It melted at 135° with decomposition.

Anal. Calcd. for $C_{17}H_{22}O_5Br_2$: Br, 34.3. Found: Br, 34.2.

Monobromoisotenulin.—Dibromoisotenulin was boiled for three minutes in a 5% methanolic solution of potassium acetate. The liquid was then diluted with three volumes of water and allowed to crystallize. The yield was quantitative, and the product was quite pure. By dissolving it in the necessary quantity of boiling methanol and adding three volumes of hot water, it was brought to a constant melting point of 213° with decomposition.

Anal. Calcd. for $C_{17}H_{21}O_5Br$: Br, 20.7. Found: Br, 21.3.

The Substance $C_{18}H_{22}O_5$ Found in One Shipment of *Helenium tenuifolium*.—In one shipment of *H. tenuifolium* obtained in 1936 it was difficult to obtain tenulin. The material obtained after the extract had stood for a long time gave roughly 60% of tenulin and 40% of another compound relatively insoluble in ethanol. Because of this property the unknown compound was readily separated from tenulin and purified by recrystallization from boiling ethanol. It consisted of long, thin, fluffy colorless rods, which melted at 233–234°. An alkoxy determination gave a value corresponding to 3.85% of methoxyl.

Anal. Calcd. for $C_{18}H_{22}O_5$: C, 65.29; H, 7.55. Found: C, 65.22, 65.36; H, 7.58, 7.55.

Dihydro Derivative of $C_{16}H_{22}O_5$.—One grain of the C_{16} compound was suspended in ethyl acetate and hydrogenated as was tenulin. The suspension gradually disappeared, and when the reaction was completed one mole of hydrogen was consumed. The filtered solution was concentrated and crystallized. The material separated as colorless rods with a melting point of 192°. Recrystalliza-

tion from boiling ethyl acetate did not change the melting point.

Anal. Calcd. for $C_{16}H_{24}O_5$: C, 64.84; H, 8.16. Found: C, 64.78; H, 8.11.

Acetyl Derivatives of the Substance $C_{16}H_{22}O_5$.—(1) A mixture of 1 g. of the C_{16} compound, 0.25 g. of dry sodium acetate, and 4 cc. of acetic anhydride was boiled for one hour. Most of the excess anhydride was then removed by distillation, and the residue was treated with water. After being stirred for some time, a semicrystalline material separated. This was dissolved in methanol and crystallized by adding water. The yield was 0.7 g. of a substance which melted at about 150°. Upon repeated recrystallization from 50% methanol the substance melted at 163°.

Anal. Calcd. for $C_{18}H_{24}O_6$: C, 64.27; H, 7.19; acetyl, 12.8. Found: C, 64.17; H, 7.30; acetyl, 10.9.

(2) When a solution of 0.5 g. of the new material in 3 cc. of pyridine and 2 cc. of acetic anhydride was allowed to stand overnight and then treated with water, crystallization occurred at once. Six-tenths gram of thick prisms, m. p. 193°, was obtained. Recrystallization of this material from its methanolic solution by adding 4 volumes of water did not change the melting point. The product consisted of thick colorless prisms many of which were hexagonal.

Anal. Found: C, 64.28; H, 7.32; acetyl, 10.8.

Summary

Helenium macrocephalum has been found to contain helenalin. *Helenium tenuifolium*, *H. elegans* and *H. badium* contain a hitherto unrecorded compound, $C_{17}H_{22}O_5$, which has been designated tenulin. Some derivatives of tenulin have been prepared which suffice to establish definitely the molecular formula given for tenulin and at the same time show that the compound obtained from *H. tenuifolium* and reported by Buehler, Whitehead, and Goodge is isotenulin. In one lot of *H. tenuifolium*, another new substance, $C_{16}H_{22}O_5$, was isolated. Several derivatives of this material have been prepared.

A diagram showing the relation between the various compounds and their origin is presented.

WASHINGTON, D. C.

RECEIVED MAY 18, 1939