Phytochemistry, 1972, Vol. 11, pp. 1101 to 1103. Pergamon Press. Printed in England.

SHORT COMMUNICATION

PSEUDOGUIANOLIDES IN *HELENIUM AUTUMNALE* FROM PENNSYLVANIA[1]

WERNER HERZ and P. S. SUBRAMANIAM

Department of Chemistry, The Florida State University, Tallahassee, Fla. 32306, U.S.A.

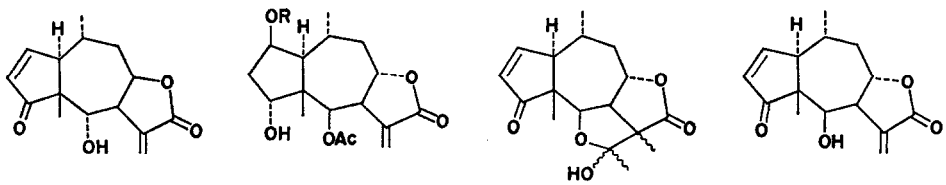
(*Received* 25 *August* 1971)

Abstract—The main sesquiterpene lactone found in collections of *Helenium autumnale* L. from Pennsylvania was not helenalin, but tenulin. Minor constituents of the flowers were mexicanin I, flexuosin A, a new lactone $C_{15}H_{20}O_4$ and the flavone hispidulin.

INTRODUCTION

Helenium autumnale L. collections of unspecified provenance are reported[2] to serve as sources of the pseudoguaianolide helenalin (I). This was verified more recently for collections of *H. autumnale* from Florida[3] and Georgia,[3] but extraction of plants from Alabama[3] gave reproducibly 2-acetylflexuosin A (IIb) and autumnolide and material from North Carolina gave the norsesquiterpene lactone dihydromexicanin C.[4]

The chemical variability of this species is now further illustrated by our study of several collections from Somerset County, Pennsylvania. Flowers and leaves were examined separately. Chloroform extraction of all parts gave, quite reproducibly, tenulin (III) as the main sesquiterpene lactone, accompanied, in the case of the flowers, by smaller quantities of mexicanin I (IV), flexuosin A (IIa) and a new sesquiterpene lactone $C_{15}H_{20}O_4$, whose polymerization on standing prevented further study. A small amount of hispidulin (5,7,4'-trihydroxy-6-methoxyflavone) was also isolated from one of the extractions.



(I) (II) a R=H b R=Ac (III) (IV)

[1] H. WAGNER, M. A. IYENGAR and W. HERZ, *Phytochem.* **11**, 446 (1972).
[2] W. HERZ, A. ROMO DE VIVAR, J. ROMO and N. VISWANATHAN, *J. Am. Chem. Soc.* **85**, 19 (1963); W. HERZ and P. S. SANTHANAM, *J. Org. Chem.* **32**, 507 (1967).
[3] W. HERZ, P. S. SUBRAMANIAM and N. DENNIS, *J. Org. Chem.* **34**, 2915 (1969).
[4] R. A. LUCAS, R. G. SMITH and L. DORFMAN, *J. Org. Chem.* **29**, 2101 (1964).

Although the sesquiterpene lactone content (chloroform extract) of the Pennsylvania collections differed qualitatively from that of the Alabama[3] and Florida–Georgia[3] collections, there was no significant difference in the flavone-*C*-glycoside content (methanol extract) from that reported previously[1] for the Southern collections (saponaretin, vitexin, iso-orientin and orientin).*

EXPERIMENTAL

Extraction of Helenium autumnale L. Powdered *H. autumnale* L. flowers, wt. 1·975 kg, collected by Dr. B. H. Braun on September 25 and on October 2 and 4, 1966 at Beaver Dam Creek along Pennsylvania Route 63 near Stoystown, Somerset County, Pennsylvania,† was extracted with *n*-hexane for 3 days, then with $CHCl_3$ and then with MeOH. The hexane extract was discarded. The $CHCl_3$ extract was worked up in the usual manner and gave 234 g of crude gum which was taken up in 500 ml of hot benzene. The solution on being allowed to stand overnight deposited 40 g of crude tenulin which was recrystallized from acetone–hexane and identified by comparison with authentic material. (m.p., mixed m.p., IR spectrum, TLC).[5] The benzene filtrate was adsorbed on a column of 1·2 kg of silicic acid (Mallinckrodt 100 mesh). Elution with benzene followed by solvents of increasing polarity gave the following results, 1 l. fractions being collected.

Fractions 1–7 (benzene) were waxy semisolids. Fraction 8 (benzene) solidified giving 1·2 g of a triterpene mixture which was not investigated further. Fractions 9–15 (benzene), 16–20 (benzene–$CHCl_3$, 4:1), 21–24 (benzene–$CHCl_3$, 3:2), 25–38 (benzene–$CHCl_3$, 1:1), 39–42 (benzene–$CHCl_3$, 1:3) and 43–46 ($CHCl_3$) eluted non-crystallizable gums showing several spots on TLC. Fractions 47–53 ($CHCl_3$) solidified on trituration with benzene. Crystallization from acetone afforded 1·4 g of mexicanin I, m.p. 258–261°, identical with an authentic specimen (IR and NMR spectrum, TLC, mixed m.p.).[6] Acetylation of 0·12 g of mexicanin I with Ac_2O–pyridine furnished 0·096 g of the acetate which melted at 202–204° after recrystallization from benzene–hexane and was identical with linifolin A[8] in all respects (IR and NMR spectrum, TLC, mixed m.p.).

Fraction 54 ($CHCl_3$) was a gum. Fractions 55–61 ($CHCl_3$–ether, 19:1) eluted 2·1 g of tenulin, 62–64 ($CHCl_3$–ether, 9:1) eluted gummy material, fractions 65–74 ($CHCl_3$–ether) solidified on trituration with ether, total yield 9·5 g of crude solid. Rechromatography over silicic acid and recrystallization from acetone–hexane–ether gave a previously unknown sesquiterpene lactone, m.p. 112–115°. Another sample of this compound, recrystallized from acetone–isopropyl ether, melted at 146° and had $[\alpha]_D^{21}$ −89·3° (c 0·94), but the IR spectra of the two samples were identical and contained bands at 1765 (γ-lactone), 1650 and 1630 cm^{-1} (two double bonds). The NMR spectrum exhibited signals at 6·25 d (3) and 5·62 d (3, exocyclic methylene group conjugated with lactone), 5·35 br superimposed on 5·25 c (vinyl proton and H—C—O of lactone), 3·80 t (1 proton), 3·5–2·6 c (complex system of four protons, reduces to two protons on exchange with D_2O) 1·78 d (1, vinyl methyl) and 1·08 ppm (methyl singlet). The UV spectrum had a maximum at 205 nm (ε 13240). *Anal.* Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63; O, 24·21. Found: C, 68·52; H, 7·41; 0, 23·81. This substance had polymerized by the time structure studies could be initiated.

Fractions 75–78 ($CHCl_3$–MeOH, 49:1) and 79–80 ($CHCl_3$–MeOH, 24:1) were gums. Fractions 81 and 82 ($CHCl_3$–MeOH, 24:1) solidified on trituration with ether. Recrystallization from EtOAc afforded 0·8 g of colorless needles, m.p. 221–222°, identical (IR and NMR spectrum, mixed m.p., TLC) with an authentic specimen of flexuosin A.[8] Further elution with more polar solvent mixtures did not furnish lactonic material.

A collection of 0·25 g of *H. autumnale* flowers made by Dr. B. H. Braun at the same spot on September 5, 6 and 18 (1965) gave 15 g of crude tenulin from the benzene extract of the crude gum and, from the chromatography of the mother liquors over silicic acid, 6 g of tenulin in the $CHCl_3$ eluate and 1·5 g of the new lactone $C_{15}H_{20}O_4$ from the $CHCl_3$ ether (19:1) eluate. Mexicanin I or flexuosin A could not be isolated in crystalline form but were present by TLC analysis. The $CHCl_3$–MeOH (33:1) eluate yielded a few mg of hispidulin (6-methoxy-5,7,4′-trihydroxyflavone),[9] m.p. 290°, identified by comparison with authentic material (mixed m.p., IR, TLC). Extraction of 1·35 kg of leaves and stems from the same collection gave

* We are indebted to Dr. M. A. Iyengar for these results. Dr. Iyengar reports a quantitative difference in vitexin content, i.e. vitexin is present only in traces in the whole plant extract from Alabama.

† Voucher No. 105,700 deposited in the herbarium of Florida State University.

[5] W. HERZ, W. A. ROHDE, K. RABINDRAN, P. JAYARAMAN and N. VISWANATHAN, *J. Am. Chem. Soc.* **84,** 3857 (1962).

[6] E. DOMINGUEZ and J. ROMO, *Tetrahedron* **19,** 1415 (1963).

[7] W. HERZ, C. M. GAST and P. S. SUBRAMANIAM, *J. Org. Chem.* **33,** 2780 (1968).

[8] W. HERZ, Y. KISHIDA and M. V. LAKSHMIKANTHAM, *Tetrahedron* **20,** 979 (1964).

[9] W. HERZ and Y. SUMI, *J. Org. Chem.* **29,** 3438 (1964).

8·2 g of tenulin in the benzene–$CHCl_3$ (1:2 and 1:3) eluates. The more polar eluates gave non-crystallizable gums which showed several spots on TLC. Rechromatography failed to furnish solid material.

A collection of 0·312 kg of powdered *Helenium autumnale* flowers collected by Dr. B. H. Braun along Wilson's Creek, Rockwood, Somerset County, Pennsylvania, on October 9 (1966), was extracted and worked up in the usual way. The crude gum, wt. 10·5 g, deposited 4 g of tenulin. Chromatography of the benzene eluate furnished 0·6 g of tenulin in the $CHCl_3$ eluate, but other sesquiterpene lactones could not be isolated in crystalline form.

Acknowledgement—We are grateful to Dr. B. H. Braun for making the collections. This study was supported by a research grant GM-05814 from the U.S. Public Health Service.

Key Word Index—Helenium autumnale; Compositae; sesquiterpene lactones, pseudoguianolides; chemotaxonomy; chemical races.