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SESQUITERPENE LACTONES.

CONSTITUENTS OF *HYMENOCLEA SALSOLA* T. AND G.

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Abstract.—*Hymenoclea salsola* T. and G. (fam. Compositae, tribe Ambrosieae) contains ambrosin (I), neoambrosin (III), coronopilin (IV), dihydrocoronopilin (V), and ilicic acid (II), as well as several additional compounds still to be characterized. The close phylogenetic relationship of *Hymenoclea* and *Ambrosia* species indicated by morphological criteria is supported by these observations.

INTRODUCTION

Hymenoclea salsola T. and G. (fam. Compositae), a perennial shrub common on the southwestern desert, is a member of the tribe Ambrosieae,[1] several other genera of which have received extensive study. *H. salsola* is rich in sesquiterpene lactones, which have now been found to include members of a closely related group which contains several compounds found in other members of the tribe as well as the new compounds described in this paper.

RESULTS AND DISCUSSION

The chief constituent of the plant, isolated in 0·4 per cent yield, is ambrosin (I), a constituent found in several species of *Ambrosia*[2,3] and in *Parthenium incanum* H.B.K.[2] and *P. hysterophorus* L.[4] A second constituent of *Hymenoclea salsola*, isolated in 0·02 per cent yield, is ilicic acid (II), previously found in *Ambrosia ilicifolia* (Gray) Payne.[5,6] The identity of the compound was established by the correspondence of its spectral properties with those reported for ilicic acid and by direct comparison (mixed m.p.) with an authentic sample.

A third compound present in *H. salsola* is the isomer (III) of ambrosin, which has been reported[7] as a product of the isomerization of ambrosin and of the dehydration of coronopilin,

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1 W. L. JEPSON, *A Manual of the Flowering Plants of California*, Assoc. Students Store, Berkeley, California, (1925); W. W. PAYNE, P. H. RAVEN and D. W. KYHOS, *Am. J. Botany* **51**, 419 (1964). The Ambrosieae are classed as a subtribe, Ambrosiinae, of the tribe Heliantheae, by P. A. Munz and D. D. Keck, *A California Flora*, University of California Press, Berkeley (1959).

2 W. HERZ, H. WATANABE, M. MIYAZAKI and Y. KISHIDA, *J. Am. Chem. Soc.* **84**, 2601 (1962).

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

4 A. ROMO DE VIVAR, E. A. BRATOEFF and T. RÍOS, *J. Org. Chem.* **31**, 673 (1966).

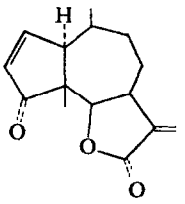
5 W. HERZ, H. CHIKAMATSU and L. R. TETHER, *J. Org. Chem.* **31**, 1632 (1966).

6 We are grateful to Professor Herz for a specimen of ilicic acid isolated from *A. ilicifolia*.

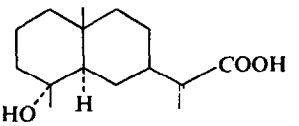
7 A. ROMO DE VIVAR, L. RODRIGUEZ-HAHN, J. ROMO, M. V. LAKSHMIKANTHAM, R. N. MIRRINGTON, J. KAGAN and W. HERZ, *Tetrahedron* **22**, 3279 (1966).

but which is hitherto unreported as a naturally occurring compound.[8] Neoambrosin (III), m.p. 126–127°, isolated in 0·07 per cent yield, has spectral properties in satisfactory agreement with those reported[7] and consistent with the structure (III) assigned to the compound. Its i.r. spectrum shows the bands (1750 cm^{-1}, broad; 1650 cm^{-1}, weak) characteristic of the combined absorption of the α-methylene-γ-lactone and the cyclopent-1-ene-4-one system. It shows only end absorption in the u.v. but on keeping III in alcoholic solution for several days a shoulder, corresponding to an absorption maximum at about 219 nm. appears, probably as a result of a slow isomerization to the conjugated cyclopentenone system of ambrosin. The NMR spectrum of neoambrosin shows a sharp singlet at 1·17 ppm (3 H ,C-5 methyl), a doublet at 1·16 ppm (3 H, J = 7; C-10 methyl), and the characteristic pair of doublets at 5·51 and 6·24 ppm (each 1 H, J = 3; α-methylene). The signal for the proton of the —CH—O grouping of the lactone appears as a sharp doublet at 4·41 ppm. (1 H, J = 9), almost identical in pattern with the signals for the same proton in ambrosin and coronopilin. The presence and position of the 1.2-double bond are shown by the signal at 5·97 ppm (1 H, triplet; C-2) and the pair of doublets centered at 2·95 ppm (2 H, $J = 8{\cdot}5, 2$). These values are in substantial agreement with those observed for the synthetic III.[7]

Catalytic reduction of neoambrosin in ethyl acetate (platinum oxide, atmospheric pressure) resulted in the absorption of one mole of hydrogen and the formation of two compounds which were separated by chromatography. One of these was identical (mixed melting point, thin-layer chromatography, i.r. and NMR spectra) with dihydroisoambrosin (IV) when compared with a specimen of IV prepared from ambrosin by hydrogenation under the same conditions (which were not identical with those used in the earlier preparation of IV).[9]



(I)



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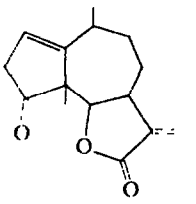

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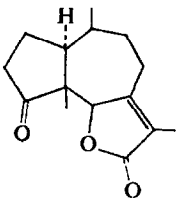

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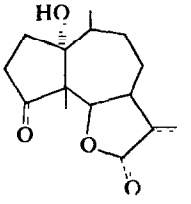

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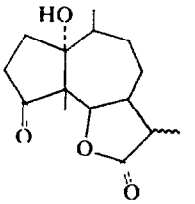

(VI)

A fourth substance, when first isolated from *H. salsola*, appeared to be coronopilin (V):[2] it has the same melting point as coronopilin; and a mixed melting point was not depressed. The NMR spectrum was so similar to that of coronopilin that it appeared at first to substanti-

[8] That (III) is not an artifact of isolation is shown by its appearance on a thin-layer chromatogram of a crude total extract of the plant.

[9] F. ŠORM, M. SUCHÝ and V. HEROUT, *Collection Czech. Chem. Commun.* **24**, 1548 (1959).

ate the conclusion that the new substance was indeed this compound, but a proton integration of the spectrum showed that the high-field region contained too many protons for the composition $C_{15}H_{20}O_4$ if the signals for the lactone methylene protons were each counted as one. The solution to these ambiguities was found when the mass spectrum was examined. Instead of the single molecular ion peak at m/e 264 (coronopilin) the spectrum showed two peaks of nearly equal intensity at m/e values of 264 and 266; and, more significantly, several pairs of prominent peaks at m/e values differing by two mass units (at 264/266 (M); 246/248 (M-18); 231/233 (M-18-15); 218/220 (M-18-28)) were found in the fragmentation pattern. This finding clarified the NMR spectrum: if the substance is a mixture of coronopilin and dihydrocoronopilin (VI) the signals for the protons of the α-methylene grouping are in reality less than one proton each, and the reason for the "excess" of protons in the high-field region is clear. Elementary analyses showed values for hydrogen that were somewhat high, a result that is also consistent with these conclusions.

Isolation of another sample of the coronopilin/dihydrocoronopilin fraction and repeated recrystallization (the compounds show identical R_f values on TLC and appear in a single fraction in column chromatography) resulted in a substantial degree of purification, but the best material shows a small molecular ion peak at m/e 264 in the mass spectrum, along with the now predominant molecular ion peak at m/e 266. The melting point of the recrystallized material was 194–197° (compared with 177–178° for coronopilin). A dihydrocoronopilin (11-epitetrahydroparthenin) melting at 189–191° has been prepared by the zinc–acetic acid reduction of coronopilin.[2] A repetition of the preparation of dihydrocoronopilin[2] yielded material that corresponded with that described but was still a mixture; but by modifying the experimental conditions, pure dihydrocoronopilin was obtained. It had m.p. 198–200°, and showed a single molecular ion peak at m/e 266. It did not depress the melting point of the natural material, and the i.r. spectra of the synthetic and natural compounds were substantially identical in all respects. Dihydrocoronopilin has not been previously found as a naturally occurring compound.

The NMR spectrum of the natural dihydrocoronopilin with m.p. 194–197° still shows a remnant of the signals for the α-methylene proton of the coronopilin impurity, and the remainder of the spectrum is nearly identical with that of coronopilin with the exception that the additional protons of the C-11 methyl group appear in the high-field region as a doublet at 1·28 ppm ($J = 7$ c/s) along with the signals for the methyl groups at C-5 (singlet, 1·15 ppm) and C-10 (doublet, 1·22 ppm). The lactone proton (—$\underset{|}{\text{C}}$H—O) appears as a doublet nearly identical in chemical shift and coupling pattern (doublet, $J = 10$ c/s) with that of the same proton of coronopilin. That no additional signal for an alcoholic (—$\underset{|}{\text{C}}$*H*OH) proton appears shows that the carbonyl group at C-4 is not reduced, and thus that the two additional mass units disclosed in the mass spectrum represent the protons added at C-11/13.

Several additional compounds (with those described above, a total of nine) have also been isolated as crystalline substances in small amounts. Further study of this complex of compounds will be resumed when more material becomes available.

The close chemical similarity demonstrated here between *H. salsola* and species of *Ambrosia* reflects a rather close phylogenetic relationship, as hypothesized below.[10] The two genera

[10] The comments in this and the following paragraph are those of Professor W. W. Payne, Department of Botany, University of Illinois, who has studied the taxonomy of the Ambrosieae and related members of the Compositae.

share all of the attributes which distinguish the Ambrosieae within the family Compositae, as well as a number of characteristics peculiar to themselves and to *Xanthium* (cockleburs). They are distinguished from one another by only one clearly defined morphological character: the nature of the appendages of the involucre of the pistillate flower heads. One may propose common ancestry for *Ambrosia* and *Hymenoclea*, a proposition centered about modification of the phyllaries (individual involucral bracts) of the seed-bearing heads as flattened, somwhat spiny processes. Modification along one line could have produced the specialized, spiny involucres of many relatively primitive *Ambrosia* species which are adapted to animal dispersal, and along another line to the flat, membranaceous phyllaries of *Hymenoclea*, adapted to wind dispersal. This suggestion is supported by the nature of the fruiting involucres of the primitive *A. deltoides*, in which the broad lower spines may function either for wind or animal dispersal.

With the above phylogenetic pattern in mind, the chemical similarities between *Hymenoclea* and *Ambrosia* may have important evolutionary implications. In particular, the occurrence in *H. salsola* of both the biosynthetically simpler ilicic acid, which occupies a position close to the origin of the probable biosynthetic pathway starting from a farnesol-derived precursor, and the more specialized pseudoguaianolide ring systems of ambrosin and its congeners, suggests that *H. salsola* may be evolutionarily rather close to the ancestral forms of the complex.

Further studies of *H. salsola* and of the closely related *H. monogyra* are proceeding and should provide further information concerning these phylogenetic questions.

EXPERIMENTAL

Extraction and Separation of Constituents

Hymenoclea salsola T. *and* G.[11] was collected near Indio, California, in March, 1966. The aerial parts were dried and ground in a Wiley mill, and a 1-kg portion was exhaustively extracted with methylene chloride at ordinary temperature. The extract was processed in the usual way,[12] the final chloroform extract yielded a yellow–brown oil (28 g) in which about thirteen distinct components could be detected by thin-layer chromatography (TLC).

The crude material was subjected to chromatography on silica gel (450 g, 3·8 × 65 cm), with successive elution with solvents ranging from benzene to chloroform–methanol (95:5). The eluates were examined by TLC and divided into fractions I–V, fraction I being that most rapidly eluted. Each fraction contained a discrete group of three or four of the total number of components of the mixture.

Neoambrosin (III)

Fraction I was chromatographed on silica gel (90 g, 2 × 30 cm) with elution with benzene and benzene–ethyl acetate mixtures. The first fraction yielded 700 mg of neoambrosin which after recrystallization from ethyl acetate–hexane had m.p. 126–127° (reported[7] m.p. 119–121°), $[\alpha]_D^{26} -66°$ (C = 2, $CHCl_3$). (Calc. for $C_{15}H_{18}O_3$: C, 73·15; H, 7·37. Found: C, 73·31; H, 7·57%.)

Dihydroisoambrosin (IV)

A. *From neoambrosin.* A solution of 0·25 g of neoambrosin in ethyl acetate was hydrogenated at atmospheric pressure with 25 mg of platinum oxide. After 50 min the uptake of hydrogen (22·5 ml, 1 mole) ceased. Examination by TLC showed that all of the neoambrosin had disappeated and two new spots were observed. Removal of the solvent and chromatography of the residual material on silica gel yielded a crystalline compound, 164–165° (reported[9] m.p. 164·5°). It had λ_{max} 217 nm ($\log \epsilon = 4·2$) and $[\alpha]_D^{26}$ 24° (C = 1·6, $CHCl_3$) (reported[9] 23·2°).

B. *From ambrosin.* Hydrogenation of 0·98 g of ambrosin in ethyl acetate (as in A) resulted in the uptake of 88·6 ml (1 mole) of hydrogen. Removal of the solvent yielded dihydroisoambrosin, m.p., after recrystallization

[11] A voucher specimen bears the number TAG-31266-HS.

[12] For typical examples of the general extraction procedure, see W. HERZ and G. HOGENAUER, *J. Org. Chem.* **26**, 5011 (1961); and T. A. GEISSMAN, *J. Org. Chem.* **31**, 2523 (1966).

from ethyl acetate–hexane, 163–165°. A mixture of the compounds prepared from neoambrosin and from ambrosin showed no depression in melting point. (Calc. for $C_{15}H_{20}O_3$: C, 72·58; H, 8·06. Found (A): 72·47; H, 7·95; (B): C, 72·56; H, 7·90%.)

Ambrosin (I)

Rechromatography of fraction II (silica gel, elution with benzene, benzene–ethyl acetate, chloroform–ethyl acetate and chloroform–ethyl acetate–acetone) yielded a series of fractions, from the earliest of which additional neoambrosin was obtained. Later fractions yielded 4·1 g of ambrosin, m.p. 143–146° (reported[13] 146°), $[\alpha]_D^{26}$ −155° (C=2, $CHCl_3$) (reported[13] −154·5°), identical (m.p., i.r., u.v., NMR) with an authentic specimen. (Calc. for $C_{15}H_{18}O_3$: C, 73·15; H, 7·37. Found: C, 73·08; H, 7·49%.)

Dihydrocoronopilin (VI)

Rechromatography of fraction III (silica gel, chloroform–ethyl acetate–acetone) yielded several fractions showing substantially only one spot on TLC, with an R_f identidal with that of coronopilin. From these fractions was obtained 250 mg of a crystalline substance (HS-5), m.p. 174–183°, raised to m.p. 194–197° by repeated recrystallization from benzene and ethyl acetate–hexane. Mixtures of the crude material with coronopilin (m.p. 178°) showed melting points in the region of 178°; no clear depression was observed. The crude and purified materials showed identical behavior on TLC. (Calc. for $C_{15}H_{20}O_4$: C, 68·16; H, 7·63; for $C_{15}H_{22}O_4$: C, 67·67; H, 8·27. Found: C, 68·01, 67·97, 68·11; H, 7·98, 7·88, 7·77%.)

The spectral properties of this material have been described and discussed in the earlier part of this paper.

Dihydrocoronopilin from Coronopilin

Coronopilin was reduced with zinc dust in acetic acid.[2] By extending the reaction time from 24[2] to 48 hr the reduction was complete, and dihydrocoronopilin (VI) uncontaminated by coronopilin was obtained. The recrystallized compound had m.p. 198–200°, and a mixture of this material with the natural dihydrocoronopilin with m.p. 194–197° showed no depression in melting point. (Calc. for $C_{15}H_{22}O_4$: C, 67·67; H, 8·27. Found: C, 67·85; H, 8·26%.)

The natural and synthetic compounds showed identical behaviour on thin-layer chromatograms.

Ilicic Acid (II)

Fractions IV and V contained nearly the same components, but when fraction V was allowed to evaporate it crystallized in part. Removal of the crystalline material and recrystallization from ethyl acetate yielded 240 mg of a compound m.p. 175–177°. It was an acid, and examination of its NMR spectrum led to the conclusion that it was ilicic acid.[5] A mixed melting point with an authentic specimen of ilicic acid[6] (m.p. 176–177°) showed no depression.

Other Components

Systematic rechromatography of the main fractions I–V resulted in the separation of a number of additional constituents, the structures of which are still to be determined.

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[13] H. Abu-Shady and T. O. Soine, *J. Am. Pharm. Assoc.* **43**, 387 (1953).