Phytochemistry, 1971, Vol. 10, pp. 1075 to 1077. Pergamon Press. Printed in England.

SHORT COMMUNICATION

DISTRIBUTION OF EUPATORIOPICRIN IN COMPOSITAE

T. A. GEISSMAN and SAMY ATALA

Department of Chemistry, University of California,* Los Angeles, Los Angeles, California 90024, U.S.A.

(*Received* 6 *August* 1970)

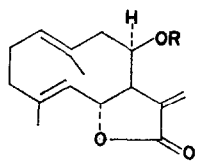
Abstract—Eupatoriopicrin, first isolated from *Eupatorium cannabinum* L. (tribe Eupatorieae), has been found to be the principal sesquiterpene lactone in *Venegasia carpesioides* DC., *Eriophyllum stachaedifolium* Lag. var. *artemisiaefolium* (Less.) Macbr., and *Chaenactis carphoclinia* Gray, and is also present in *Chaenactis douglasii* (Hook.) H. and A., all members of the tribe Heleniae.

RESULTS AND DISCUSSION

EUPATORIOPICRIN (I) was first isolated from *Eupatorium cannabinum* L.[1] The compound is a β-8-hydroxygermacranolide[2] esterified with 2-hydroxymethyl-4-hydroxy-2-butenoic acid. Eupatolide, the unesterified 8-hydroxygermacranolide, is epimeric at C-8 with deacetyltulipinolide, IV, a constituent of *Liriodendron tulipfera* (Magnoliaceae).[3]

Examination of several representatives of the tribe Heleniae has disclosed that eupatoriopicrin is of relatively widespread occurrence. It is present in substantial amounts (about 0·5–1·0% dry wt.) in *Venegasia carpesioides* DC., *Eriophyllum stachaedifolium* Lag. var. *artemisiaefolium* (Less.) Macbr. and *Chaenactis carphoclinia* Gray. Its presence as a prominent constituent in *Chaenactis douglasii* (Hook.) H. and A. was established by TLC of extracts of the latter. Eupatoriopicrin is readily distinguished on TLC by the fact that upon warming a plate sprayed with sulfuric acid the spot becomes a characteristic green-blue color before further darkening.

The isolation of eupatoriopicrin can be accomplished with ease, for it often crystallizes at once from an aqueous-alcholic extract of the total chloroform extractives of the plant. In the course of the present studies eupatoriopicrin was hydrolyzed to the known eupatolide (II)[1] and this was converted to the crystalline acetate (III), which has been described as the naturally occurring epitulipinolide.[3]



I, R=COC(CH$_2$OH)=CHCH$_2$OH
II, R=H
III, R=COCH$_3$
IV, R=H (OR-α)

* Contribution No. 2674 from the Department of Chemistry, University of California, Los Angeles, California, U.S.A.

[1] L. DOLEJS and V. HEROUT, *Coll. Czech. Chem. Commun* **27**, 2654 (1962).
[2] M. DRODZ, M. HOLUB, unpublished observations in this laboratory, to be published.
[3] R. W. DOSKOTCH and F. S. EL FERALY, *J. Pharm. Sci.* **58**, 877 (1969).

The configuration of the esterifying acid has now been established by partial hydrogenation and hydrolysis of I. The principal product of the hydrogenation was α-methylbutyric acid, accompanied by varying amounts of tiglic acid. That the tiglic acid was not formed as a result of isomerization of angelic acid or an angelate during hydrolysis was established by observing that the NMR spectrum of I showed the proton at C-3 of the esterifying acid at the same chemical shift as that of the corresponding proton in tiglic acid. Angelic and tiglic acids are clearly distinguished in the chemical shift of this proton.[4]

The occurrence of esters of the simple hydroxygermacranolide (II) in several sections of Compositae as well as in Magnoliaceae can be reconciled with its position close to the presumed biosynthetic origin of compounds of the class; but the presence of eupatoriopicrin, which possesses the unusual esterifying acid, suggests a close affinity between the disparate species in which it is found that is not apparent in their dissimilar morphological features.

EXPERIMENTAL

Plant Material

The plants used in this study were as follows: *Venegasia carpesioides*: Collected in the Santa Monica mountains, Los Angeles; voucher TAG-51466-VC. *Eupatorium cannabinum*: Collected near Starnberg, Germany; voucher TAG-71069-SP. *Eriophyllum stachaedifolium* var. *artemisiaefolia*: Collected near San Simeon, California; voucher TAG-10067-MB. *Chaenactis carphoclinia* and *C. douglasii*: Collected and authenticated by Mr. R. J. Barr, American West Botanical Co., El Paso, Texas.

Isolation of eupatoriopicrin (I). (A) From *Venegasia carpesioides*: Dried leaves (500 g) of the flowering plant were extracted with $CHCl_3$. The residue remaining after removal of the solvent was extracted with 200 ml of ethanol–water (1:4) and the clarified yellow aqueous solution extracted with $CHCl_3$, from which was obtained 10·0 g of a brownish-yellow syrup. This largely crystallized on standing. Trituration with EtOAc–pentane (2:1) gave 3·6 g of nearly colorless crystals of I. Reworking of the filtrates and washings yielded another 1·9 g, making the total yield 5·5 g (1·1%). Crystallized from EtOAc–pentane the compound had m.p. 155–156° and gave a single spot on TLC.

In another experiment, 560 g of plant material gave 4·46 g (0·8%).

Anal. Found, C, 66·24; H, 7·32. $C_{20}H_{26}O_6$ requires C, 66·28; H, 7·23.

(B) From *Eupatorium cannabinum*: Extraction of 1·2 kg of *E. cannabinum* with $CHCl_3$ and removal of the solvent gave a black-green tar that was dissolved in 200 ml of ethanol. After the addition of 500 ml of hot water the tarry deposit was separated and the aqueous solution clarified by filtration (celite, charcoal). Crystals began to separate from the filtrate, and after cooling, the product was collected. There was obtained 6·30 g of crude I which was purified by a passage through a silica gel column ($CHCl_3$). The product was recrystallized from EtOAc–light petroleum and had m.p. 156–158°. Extraction of the aqueous filtrate with $CHCl_3$ and chromatography of the extracted material over silica gel provided 2·52 g of additional I. The total yield was 8·82 g (0·73%).

(C) From *Eriophyllum stachaedifolium* var. *artemisiaefolia*: the plant was processed in the manner described above; 24 g of dry plant material yielded 125 mg of I, which crystallized spontaneously without the necessity for column chromatographic purification.

(D) The isolation of I from *Chaenactis carphoclinia* was carried out in the same way.

(E) The presence of I in *Chaenactis douglasii* was recognized by TLC.

Mixed m.ps of the specimens of I found in these various plants showed no depression.

Eupatolide (II). Eupatoriopicrin (2·2 g) was saponified by gentle warming in 40 ml of 1N KOH until solution was complete. Acidification of the solution with acetic acid gave a clear, colorless solution from which crystals separated on cooling and scratching. The product (1·52 g) was not the lactone but the corresponding hydroxy acid, for it remained at the origin of a TLC plate (silica gel) developed with benzene–acetone (3:1). Conversion to the lactone takes place with great ease; recrystallization from aq. acetic acid, or warming an ethanolic solution containing a trace of HCl gave the lactone, which behaved as expected on TLC. The pure compound had m.p. 181–183°.[1]

Eupatolide acetate (III). Acetylation of eupatolide with Ac_2O–pyridine in the usual way yielded the acetate, colorless prisms from ether–pentane, m.p. 84° (reported 91–92°).[3] The NMR spectrum was in complete accord with the assigned structure and agreed in all details with that of epitulipinolide.[3]

[4] M. D. NAIR and R. ADAMS, *J. Am. Chem. Soc.* **82,** 3786 (1960).

Hydrolysis of partially reduced I: Tiglic Acid. Hydrogenation of eupatoriopicrin (3·3 g) at room temp. in dimethylformamide in the presence of 10% Pd-C was interrupted before hydrogen uptake was complete. Saponification of the reduced material and isolation of the acidic fraction in the usual way afford a product which was found by NMR spectroscopy to be a mixture of tiglic and α-methylbutyrica acids. A similar hydrogenation experiment carried out at 0–5° gave an acid mixture richer in tiglic acid. Spectral (NMR) comparisons were made with the mixture of acids derived from I and mixtures of authentic tiglic and α-methylbutyric acids. The spectra were identical in all details. The signal for the $=CHCH_2OH$ proton in the NMR spectrum of I appeared at 6·95 ppm; that for the corresponding proton in tiglic acid was seen at 6·99 ppm.

Acknowledgments—One of us (T.A.G.) wishes to express his thanks to Professor L. Hörhammer, Institut für Pharmazeutische Arzneimittellehre, University of Munich, Germany, for providing the facilities of his laboratory for part of this study. Some of the costs of this work were provided by a grant from the U.S. Public Health Service, GM-14240. Analyses are by Miss Heather King.