Tumor-Inhibitory Agent from *Hyptis emoryi* (Labiatae)

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Abstract □ The extract of *Hyptis emoryi* (Labiatae) has shown tumor-inhibiting properties in the 5WA16 test system of the Cancer Chemotherapy National Service Center. By means of melting point, mixed melting point, IR, mass spectrometry, elemental analysis, and preparation of derivatives, the agent responsible for this action was shown to be betulinic acid.

Keyphrases □ *Hyptis emoryi* (Labiatae)—identification of betulinic acid as tumor-inhibiting constituent □ Betulinic acid—identified as the tumor-inhibiting constituent of *Hyptis emoryi* □ Antitumor agents—betulinic acid isolated from *Hyptis emoryi*, identified as the tumor-inhibiting constituent □ Medicinal plants—isolation and identification of betulinic acid from *Hyptis emoryi* as antitumor agent

As a result of the continuing search for plants having tumor-inhibiting constituents from the Southwestern United States and Mexico, it was found that the chloroform extract of the aboveground parts of *Hyptis emoryi* Torr. (Labiatae)[1] was active against Walker carcinoma 256 (intramuscular) tumor system (5WA16) of the Cancer Chemotherapy National Service Center. Activity was detected in Sprague rats at a level of 36% T/C at 600 mg./kg. and of 47% T/C at 400 mg./kg. Activity in this system is defined as a percent T/C value of less than 60 in a satisfactory dose–response test (1).

A preliminary examination of the chloroform extract revealed that terpene-like compounds were apparently responsible for the tumor-inhibitory effect. Separation and subsequent testing of the two isolated (TLC) terpenes (A and B) showed that Compound A was the active constituent. The resolution of these two compounds was effected by solvent extraction followed by silica gel G dry column chromatography, using chloroform–dioxane (9:1) as the eluting solvent system. Compound A, which demonstrated activity of 15% T/C at 500 mg./kg. and of 52% T/C at 300 mg./kg., was identified as betulinic acid by its melting point, mixed melting point, IR, mass spectrometry, and elemental analysis. It was further characterized by preparing a methyl ester and acetate derivative.

EXPERIMENTAL

Isolation of Active Fraction—The stems, leaves, and flowers (10 kg.) of *H. emoryi* were dried, ground, and extracted in an extractor (Lloyd) with chloroform for 48 hr. The solvent was changed after 24 hr. and the extraction was continued. The combined chloroform extract, after removal of the solvent in air, yielded a residue weighing 280 g. A portion (25 g.) of this residue was stirred mechanically with acetone (1 l.) for 2 hr. at ice bath temperature and then filtered. The acetone-insoluble residue (which did not contain Compound A as shown by TLC: silica gel G, chloroform–acetone, 9:1) was inactive and discarded. The acetone extract, after removal of the solvent in air, was stirred mechanically with petroleum ether (b.p. 60–80°) (2 × 800 ml.) for 3 hr. at ice bath temperature and filtered, and the filtrate was discarded. The petroleum ether-insoluble material (12.5 g.) showed (TLC) essentially a mixture of A and B.

Isolation of Betulinic Acid—The above mixture was dissolved in acetone–chloroform (1:1 v/v) and adsorbed on silica gel G (100 g.), and the solvent was removed. The resulting silica gel G coated with the extract was then placed on the top of a column (138 × 6 cm.) packed with silica gel G (1250 g.) and eluted with chloroform–dioxane (9:1). One hundred 20-ml. fractions were collected. Based on TLC analysis, Fractions 9–13 were combined to give crude betulinic acid (1.12 g.). Repeated crystallization of this material from acetone–tetrahydrofuran (activated charcoal[2]) and finally from excess methanol (activated charcoal[2]) yielded pure betulinic acid in colorless long needles, m.p. 291–293°, which was not depressed by a mixed melting point with an authentic sample[3]. The compound retained 1 mole of methanol even after drying at 120°. The IR spectra were identical. The compound showed specific rotation $[\alpha]_D$ of +6.77° in pyridine (c, 0.3840) [lit. (2) $[\alpha]_D^{22}$ +7.79° in pyridine] and no selective absorption in the UV region, and it gave a positive tetranitromethane test. Mass spectrometry indicated a parent peak of 456.

Anal.—Calc. for $C_{30}H_{48}O_3 \cdot CH_3OH$: C, 76.22; H, 10.65. Found: C, 75.92; H, 10.73.

Preparation of Methyl Betulinate—Betulinic acid (140 mg.) was dissolved in dry ether (45 ml.), and an ethereal solution of diazomethane was added in portions at 0° for 15 min. After the addition, the reaction mixture was allowed to stand at room temperature for 2 hr. Methyl betulinate was crystallized from methanol–chloroform in colorless needles, m.p. 215°, which was not depressed by mixing with an authentic sample[3,4]. The IR spectra were superimposable, showed specific rotation $[\alpha]_D$ of −12.21° in chloroform (c, 0.4260), and gave a positive tetranitromethane test.

Anal.—Calc. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.63; mol. wt., 470. Found: C, 78.84; H, 10.45; *m/e* 470.

Preparation of Betulinic Acid Acetate—To 150 mg. of betulinic acid were added pyridine (1.5 ml.) and acetic anhydride (4 ml.). The compound obtained had a melting point of 283–285° (methanol–chloroform) [lit. (2) m.p. 287–289° dec.] and IR maxima (CCl_4) at 2.78, 2.92, 6.11, and 8.09 μ. It also gave a positive tetranitromethane test.

Anal.—Calc. for $C_{32}H_{50}O_4$: C, 77.11; H, 10.04; mol. wt., 498. Found: C, 76.51; H, 10.00; *m/e* 498.

REFERENCES

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(2) V. Bruckner, J. Kovacs, and I. Koczka, *J. Chem. Soc.*, **1948**, 948.

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[1] The plant was collected in Yuma County, Ariz., during May 1969. Identification was confirmed by Robert J. Barr, College of Pharmacy, and Dr. Charles Mason, Botany Department, University of Arizona, Tucson. A reference specimen was also deposited in the University of Arizona Herbarium.

[2] Norite.

[3] The authors are grateful to Dr. R. P. Rastogi, Central Drug Research Institute, Lucknow, India, for providing this sample.

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