Tumor Inhibitors II: Constituents and Antitumor Activity of *Sarracenia flava*

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Abstract □ The chloroform extracts of the roots and leaves of *Sarracenia flava* showed antitumor activity against lymphocytic leukemia P-388. Lupeol was identified as one constituent responsible for this activity. β-Sitosterol and α-amyrin were isolated from the hexane extract of the roots.

Keyphrases □ *Sarracenia flava*—constituents isolated, identified, and screened for antitumor activity □ Lupeol—isolated from *Sarracenia flava*, screened for antitumor activity □ β-Sitosterol—isolated from *Sarracenia flava*, screened for antitumor activity □ α-Amyrin—isolated from *Sarracenia flava*, screened for antitumor activity □ Antitumor activity—constituents isolated from *Sarracenia flava* screened

The chloroform and aqueous extracts of the insectivorous (1, 2) plant *Sarracenia flava* (golden trumpet) were reported (3) to possess antitumor activity against human epidermoid carcinoma of the nasopharynx (KB). Betulin was identified as one constituent responsible for this activity.

The antitumor activity of *S. flava*[1,2] in the lymphocytic leukemia P-388 test system is now discussed, and the isolation of several constituents from the roots of this plant is reported.

RESULTS AND DISCUSSION

Preliminary screening[3] data of the activity of the roots of *S. flava* against lymphocytic leukemia P-388 indicated that the crude hexane and ethanol extracts were inactive (T/C < 125%). However, further fractionation of the roots yielded the alkaline-soluble Fraction H, as previously described (1). Removal of betulinic acid *via* crystallization yielded a fraction giving a T/C value of 138% at a dose level of 400 mg/kg against lymphocytic leukemia in BDF_1 mice. Sequential extraction of the pitchers of *S. flava* with hexane and benzene resulted in a fraction (benzene extract from which betulinic acid was removed by crystallization) with a T/C value of 150% at a dose level of 50 mg/kg. Thus, significant activity has been demonstrated against lymphocytic leukemia. Further purification of the benzene extract of the leaves is expected to result in enrichment of activity, since the active components (betulin and lupeol) present in the roots occur in this active extract in only trace quantities.

Utilization of the previously reported (3) procedure resulted in the isolation of the sterol β-sitosterol (M^+ = 414) and the triterpene α-amyrin (M^+ = 426) from the hexane fraction. α-Amyrin and α-amyrin acetate were identical with authentic samples based on NMR, mass, and IR spectra and melting points. The melting points and IR, mass, and NMR spectra of β-sitosterol and its acetate were also identical with authentic samples. The Cancer Chemotherapy National Service Center[3] indicated that β-sitosterol and α-amyrin possess only marginal activity in the SWA 16 tumor systems.

Repetitive chromatography of chloroform-soluble Fraction B (3), after removal of betulinic acid by fractional crystallization, resulted in the isolation of the known tumor (3, 4) inhibitor lupeol in addition to betulin, which was reported (3) previously. The mass spectrum of lupeol showed an M^+ peak at 426 with major fragments at *m/e* 218, 207, and 189, indicative of the lupene skeleton. Identification was confirmed by a comparison of the mixed melting point and IR spectrum with those of an authentic sample. Lupeol is a known antitumor agent (4) and has shown activity in Sprague rats against the Walker 256 intramuscular tumor system (T/C = 39% at doses of 200 mg/kg).

EXPERIMENTAL

Column Chromatography Separation of Fraction A (3)—The crude hexane extract (Fraction A) (41 g) was dissolved in petroleum ether (100 ml) and placed on the chromatography column (4.5 cm diameter) with 600 g of alumina (neutral, activity I) as an adsorbent. The column was eluted with, in order, petroleum ether, 10% ether–petroleum ether, 20% ether–petroleum ether, 50% ether–petroleum ether, and methanol. After the collection of 1-liter fractions, the solvent was removed *in vacuo*. The separations were monitored by TLC, using acetone–benzene–chloroform (1:2:17) as the eluting system.

Isolation and Identification of α-Amyrin—The waxy compound from the petroleum ether eluate was dissolved in hexane (50 ml) and passed through a chromatography column (2 cm diameter, neutral alumina, activity I, 100 g). The column was eluted with hexane (1300 ml) and 5% ether–hexane (1700 ml). The eluate was collected in 100-ml fractions. Combined fractions 24–31, which were eluted with 5% ether–hexane, contained mainly one spot on TLC (R_f 0.153 in 10% ether–petroleum ether). Crystallization from ether–petroleum ether gave white leaflet crystalline α-amyrin, mp 171–175°, 2.45 g (0.06%). Recrystallization of α-amyrin from ethanol yielded needle crystals, mp 185–186° [lit. (5) mp 186°].

The melting point of lupeol was undepressed on admixture with an authentic sample of α-amyrin[4]. The IR, NMR, and mass spectra were in complete agreement with those of an authentic sample of α-amyrin. The IR, NMR, and mass spectra of α-amyrin acetate were also in complete agreement with those of an authentic sample of α-amyrin acetate prepared by standard procedures (3).

Isolation and Identification of β-Sitosterol—When the 20% ether–petroleum ether eluate was concentrated to about 20 ml and filtered and the residue was recrystallized from hot chloroform–methanol (1:2), 220 mg (0.005%) of white needle crystalline β-sitosterol, mp 135–136° [lit. (6) mp 137°], was obtained. The melting point of β-sitosterol was undepressed on admixture with an authentic sample[5]. The IR, NMR, and mass spectra of β-sitosterol were identical with those of the authentic sample. The IR, NMR, and mass spectra of β-sitosterol acetate were also in complete agreement with those of an authentic sample. Standard procedures (3) were employed in the preparation of β-sitosterol acetate.

Isolation of Lupeol—Fifty grams of Fraction E (3) was extract-

[1] The plant material used in this investigation was identified as *Sarracenia flava* L. (Sarraceniaceae) by Dr. Sidney McDaniel, Department of Botany, Mississippi State University. A voucher (preserved) specimen (SM-16,702) representing material collected for this investigation is available for inspection at the Herbarium of the Department of Botany, Mississippi State University.

[2] Collected 14 miles west of Panama City, Fla., in June 1973.

[3] Cancer Chemotherapy National Service Center, Bethesda, MD 20014, 1970.

[4] Pfaltz and Bauer.

[5] Aldrich Chemical Co.

ed seven times with 250 ml of 20% benzene–petroleum ether. The solvent was removed *in vacuo* from the combined extracts to yield 11.2 g of material. The crude extract was dissolved in 50 ml of benzene and placed on a chromatography column (2.5 cm diameter × 55 cm long, neutral alumina, activity I). The column was eluted with, in order, petroleum ether, 20% benzene–petroleum ether, 40% benzene–petroleum ether, and pure benzene (200 ml/fraction).

Fractions 1–7 of the 40% benzene–petroleum ether elution were purified by preparative TLC [9.75 mm thickness, silica gel GF-254, acetone–benzene–chloroform (1:2:17) as the solvent, and chloroform as the extracting solvent]. Lupeol was obtained as a white crystalline solid (11 mg), mp 206–208° [lit. (7) mp 215–216°]. The IR and mass spectra of lupeol were completely in agreement with those of authentic lupeol.

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Potential Organ or Tumor Imaging Agents XV: Radioiodinated Phenytoin Derivatives

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Abstract □ Three radioiodinated phenytoin analogs were synthesized, and tissue distribution studies were conducted in rats. Except for a greater retention of radioactivity following administration of the radioiodinated derivatives, the tissue distribution patterns were qualitatively similar to those found for ^{14}C phenytoin. In nearly all cases, the adrenals, heart, kidneys, and liver displayed the greatest capacity to retain radioactivity. The high uptake of radioactivity observed for the thyroid was attributed to *in vivo* metabolic deiodination of the radioiodinated derivatives.

Keyphrases □ Phenytoin—radioiodinated derivatives, potential tumor imaging agents, tissue distribution studies, rats □ Radiopharmaceuticals—radioiodinated phenytoin derivatives, potential tumor imaging agents, tissue distribution studies, rats □ Tissue distribution—radioiodinated phenytoin derivatives, potential tumor imaging agents, rats □ Tumor imaging agents—radioiodinated phenytoin derivatives, tissue distribution studies, rats

In the search for radiopharmaceuticals that will selectively concentrate in various tumors and organs, several reports concerning the tissue distribution of phenytoin were of interest. Although early studies with this drug focused on only a few tissues, the concentration of phenytoin in the liver, kidneys, skeletal muscle, and brain was found to exceed plasma levels (1–3). Moreover, higher concentrations of phenytoin were reported in human primary brain tumors than in normal brain tissue (4).

More recently, autoradiographic studies (5) revealed that the concentration of phenytoin in the brain of cats was one to three times greater than in plasma at 0.5–6 hr following an intravenous dose. Similar studies in pregnant mice (6) revealed an intense and persistent concentration of phenytoin in the heart. This finding suggested that the antiarrhythmic action of phenytoin may be related to its affinity for myocardial tissue.

Several ^{11}C-hydantoins were synthesized (7), and an accumulation of radioactivity in the liver and, to a lesser extent, heart was noted within 5–10 min following intravenous administration to dogs. The uptake was not specific for cardiac muscle, however, since skeletal muscle contained a comparable concentration of radioactivity. Radioactivity was also high in the pancreas and mesenteric fat.

The purposes of the present study were to synthesize several radioiodinated analogs of phenytoin and to evaluate their potential utility as brain or myocardial scanning agents. Although several radiopharmaceuticals are currently employed in nuclear medicine for localization of brain tumors, these agents do not concentrate selectively in brain tissue or tumors. Instead, their selective localization arises from their passive diffusion from the bloodstream to the tumor as a result of a breakdown in the blood–brain barrier (8). As a result, positive uptake of radiopharmaceuticals is shown by hematomas, abscesses, and infarcts as well as tumors. A more tumor-specific agent is needed. Moreover, no radiopharmaceutical is currently available for imaging the myocardium, but several are undergoing evaluation for this purpose.

Since none of the elements of phenytoin has a useful γ-emitting nuclide, it was thus necessary to intro-