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Antitumor Agents from *Alnus oregona* (Betulaceae)

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Abstract □ The chloroform extract of *Alnus oregona* showed antitumor activity against the Walker 256 (5WA16) tumor system. Lupeol and betulin were identified as the two constituents responsible for this activity.

Keyphrases □ *Alnus oregona* (Betulaceae)—isolation and identification of two antitumor constituents, lupeol and betulin □ Lupeol—antitumor agent identified from *Alnus oregona* □ Betulin—antitumor agent identified from *Alnus oregona* □ Antitumor activity—isolation and identification of lupeol and betulin as antitumor constituents from *Alnus oregona*

During the routine screening of Southwestern plants for potential antitumor activity, the chloroform extract of the stembark of *Alnus oregona* Nutt. showed significant antitumor activity in Sprague rats against the Walker 256 intramuscular tumor system (5WA16)[1]. Activity in this system is defined as a percent T/C value of less than 60 in a satisfactory dose–response test (1). The plant was collected in California[2].

Triterpenes belonging mainly to the taraxane and lupane series have been isolated from various species of *Alnus*. Taraxerol, taraxerone, lupeol, lupenone, betulin, and betulinic acid as well as other triterpene compounds have been isolated from *A. glutinosa* (2, 3), *A. incana* (4, 5), *A. virdis* (6), *A. barbata* (7), and *A. subcordata* (8). However, a search of the literature failed to reveal any chemical investigation of *A. oregona*.

RESULTS AND DISCUSSION

Because of its chemical complexity, the chloroform extract was separated into six fractions by column chromatography using partially deactivated alumina (Table I). Fraction E, which consisted essentially of β-sitosterol, was not screened further since the Cancer Chemotherapy National Service Center has indicated that this compound showed marginal activity in the 5WA16 tumor system. Only Fractions C and F showed significant activity (Table II).

Fractions C and F contained essentially single components. Fraction C, upon recrystallization from chloroform–methanol, yielded a crystalline compound, m.p. 210–212°. Its mass spectrum showed an M^+ peak at 426 with major fragments at *m/e* 218, 207, and 189. These were indicative of the lupene skeleton (9). The NMR spectrum of the compound indicated vinyl protons at δ 4.66 and 4.75 (d) as well as 3α-H at δ 3.28 (m), further proof that the compound was probably lupeol. Identification was confirmed by melting point, optical rotation, and IR of the compound as well as of the acetate and benzoate. The IR of the latter was superimposable with the IR of an authentic sample of lupeol benzoate[3].

The crystalline compound isolated from Fraction F (m.p. 252–254°) was identified as betulin. The mass spectrum showed an M^+ peak at 442, and the NMR spectrum indicated the appropriate signal for vinyl protons. The IR spectra of the compound and its diacetate were superimposable with authentic samples[4].

Table I—Alumina Chromatography of Crude Extract

Fraction	Eluent		Components
A	Hexane to hexane–benzene	(3:1)	—
B	Hexane–benzene	(6:4)	—
C	Hexane–benzene	(1:1)	Lupeol
D	Hexane–benzene	(1:1)	—
E	Benzene		β-Sitosterol
F	Benzene–chloroforn	(3:1)	Betulin

Table II—Biological Activity against 5WM Tumor System

Compound	Dose, mg./kg.	Survivors	Percent T/C (1)
Crude extract	200	4/4	28
Fraction C	400	4/4	22
	200	4/4	46
Fraction F	400	4/4	39
Lupeol	200	4/4	39
Betulin	600	4/4	13
	400	3/4	26

EXPERIMENTAL

Extraction—Twelve kilograms of the dried stembark of *A. oregona* was extracted with 21 l. of chloroform in an extractor (Lloyd). The extract, after filtration and removal of the chloroform, weighed 382 g.

Column Chromatography—Neutral alumina (3.8 kg., activity III) was packed in a glass column (64 × 10.5 cm.), using *n*-hexane

[1] Cancer Chemotherapy National Service Center, Bethesda, MD 20014

[2] By the U. S. Department of Agriculture.

[3] Obtained through the courtesy of Dr. Jack L. Beal, College of Pharmacy, Ohio State University, Columbus, Ohio.

[4] Authentic specimens of betulin and its diacetate were obtained through the courtesy of Dr. C. Steelink, Department of Chemistry, University of Arizona, Tucson, AZ 85721

as the chromatographic solvent. Then 200 g. of the chloroform extract of *A. oregona* was adsorbed onto 500 g. of alumina III, and the dried pulverized mixture was placed on top of the column. The column was eluted first with hexane and then with solvents of increasing polarity. Each fraction was examined by TLC, silica gel G, using chloroform–benzene–ethyl acetate (4:8:1) as the solvent system and ceric sulfate as the developing reagent. The fractions showing the same materials on TLC were combined into six groups.

Isolation of Lupeol—Fraction C yielded a large precipitate of crystals. After repeated crystallizations from chloroform–methanol, 14 g. of a colorless crystalline compound was obtained. Its melting point was 210–212°, $[\alpha]_D$ +25°, compared with the values for lupeol of 215° and $[\alpha]_D$ +27° (10).

Preparation of Lupeol Acetate—Lupeol (0.138 g.) was dissolved in 2.0 ml. of pyridine, and 2.0 ml. of acetic anhydride was added to the mixture. The mixture was left standing overnight at room temperature. It was then poured over crushed ice. The precipitated compound was collected and washed thoroughly with water until free of acetic acid and pyridine. After drying under vacuum, the compound was recrystallized twice from chloroform–methanol. Solid needles of crystals, m.p. 211–212° and $[\alpha]_D$ +33°, were obtained. The reported values for lupeol acetate were m.p. 218° and $[\alpha]_D$ +47° (10).

Preparation of Lupeol Benzoate—A mixture of isolated lupeol (200 mg.), pyridine (2.0 ml.), and benzoyl chloride (2.0 ml.) was refluxed for 1 hr. It was then cooled and poured over crushed ice. The mixture was refrigerated for 1 day. The precipitated compound was filtered off and then redissolved in ether. The ethereal solution was successively washed three times each with 5% sodium bicarbonate, 5% hydrochloric acid, and water. After drying the washed ethereal layer over anhydrous sodium sulfate, it was filtered and the ether was allowed to evaporate at room temperature. The residue, upon repeated crystallization from chloroform–ethanol, gave long platelike crystals, m.p. 258–260°, $[\alpha]_D$ +56° [lit. (10) m.p. 273–274°, $[\alpha]_D$ +61°]. The IR spectrum of this compound (KBr pellet) and that of an authentic sample of lupeol benzoate were superimposable.

Isolation of Betulin—Fraction F yielded a large quantity of a crystalline compound. This material was purified by recrystallization from chloroform and then from ethanol. Solid, thin, rodlike crystals, m.p. and mixed m.p. 253–254°, $[\alpha]_D$ + 18° [lit. (11) m.p. 251–252°, $[\alpha]_D$ +20°] were obtained. The IR spectrum of the isolated betulin (KBr pellet) and that of an authentic sample of betulin were in complete agreement.

Preparation of Betulin Diacetate—Betulin (0.3 g.), pyridine (0.3 ml.), and acetic anhydride (3.0 ml.) were refluxed for 1 hr. After cooling the reaction mixture, a precipitate was obtained. The material, after repeated crystallization from ethanol, gave bladelike crystals, m.p. 222–225°, $[\alpha]_D$ +24° [lit. (10) m.p. 223–224°, $[\alpha]_D$ +22°]. An authentic sample of this compound gave an identical IR spectrum (KBr pellets).

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Antibacterial Activity of Certain 3-Substituted Benzothiazoline-2-thiones

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Abstract □ Preliminary antibacterial screening results for 18 compounds are provided. Sixteen compounds exhibited some degree of activity.

Keyphrases □ Benzothiazoline-2-thiones, 3-substituted—18 compounds screened for antibacterial activity against four organisms □ Antibacterial activity—18 3-substituted benzothiazoline-2-thiones screened against four organisms □ 3-Aminomethylbenzothiazoline-2-thiones, substituted—18 compounds screened for antibacterial activity against four organisms

Antibacterial (1–4), antispasmodic (5), and antitubercular (6, 7) properties have been demonstrated by benzothiazoline-2-thione and some of its derivatives. Many of these compounds were effective against *Candida albicans* and bacterial strains resistant to penicillin (4). These observations led us to synthesize a series of 3-substituted benzothiazoline-2-thiones (I). The synthesis of I was reported elsewhere (8). The present article describes the evaluation of compounds of type I against four organisms by the agar diffusion technique (9).

EXPERIMENTAL

The agar medium was inoculated with 1 ml. of 24-hr.-old culture of the test organism. Filter paper disks (5-mm. diameter) saturated with the solution of the test compound (20 mg./ml. in ethanol) were