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Tumor Inhibitory Agents from *Vauquelinia corymbosa* (Rosaceae)

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Abstract □ The chloroform extract of *Vauquelinia corymbosa* Correa has shown activity against the P-388 lymphocytic leukemia test system. The constituents responsible for this activity were identified as uvaol, ursolic acid, and betulinic acid. Their identity was proven by melting point; mixed melting point; elemental analysis; IR, PMR, and mass spectra; and preparation of derivatives.

Keyphrases □ *Vauquelinia corymbosa*—chloroform extract of leaves and stems, uvaol, ursolic acid, and betulinic acid isolated, screened for antitumor activity □ Antitumor agents, potential—uvaol, ursolic acid, and betulinic acid isolated from leaves and stems of *Vauquelinia corymbosa*, screened

As a result of the continuing search for plants having tumor-inhibiting constituents, the ethanol extract of the leaves and stems of *Vauquelinia corymbosa* Correa (Rosaceae)[1] was found to have inhibitory activity toward the P-388 lymphocytic leukemia test system (3PS)[2].

DISCUSSION

The chloroform extract, obtained from an ethanol extract by partition between chloroform and water, was subjected to column chromotography and yielded three pure components. These were identified as uvaol, betulinic acid, and ursolic acid by means of their melting points; mixed melting points; elemental analysis; mass, PMR, and IR spectra; and preparation of derivatives.

In the 3PS test system, uvaol demonstrated an activity of 125% test/control (T/C) at both 100 and 200 mg/kg, betulinic acid demonstrated an activity of 135% T/C at 100 mg/kg and 140% T/C at 50 mg/kg, and ursolic acid demonstrated an activity of 125% T/C at 50 mg/kg. Activity in the 3PS test system is defined as an increase in the survival of treated animals over that of controls, resulting in a test/control value greater than or equal to 125% (1).

EXPERIMENTAL[3]

The dried leaves and stems (6 kg) of *V. corymbosa* were ground and exhaustively extracted in a Lloyd-type extractor with petroleum ether. The marc was air dried and extracted similarly with ethanol. After removal of the solvent in air, the residue (750 g) was partitioned between chloroform and water (1:1), using 1 liter of each phase for each 125 g of alcohol residue.

The chloroform phases were combined and the solvent was removed in air. The residue (207 g) was chromatographed over neutral alumina (5.3 kg) (Brockmann activity grade III), eluting with solvents of increasing polarity. Three crystalline fractions were obtained: uvaol, betulinic acid, and ursolic acid (in order of elution).

Ursolic Acid—Elution with benzene–chloroform (1:1) gave 42.5 g of a semicrystalline solid. Two recrystallizations from ethanol provided pure material, mp 288–291°, $[\alpha]_D^{25}$ + 60°. An authentic specimen[4] of ursolic acid had a melting point of 285–287° and an $[\alpha]_D^{25}$ of + 62°; a mixture of the two samples had an undepressed melting point. PMR, IR, and mass spectra were identical. The methyl ester had a melting point of 168–170° [lit. (2) mp 166–168°]; the acetate had a melting point of 288–293° [lit. (3) mp 288–290°]; and the methyl ester acetate had a melting point of 247–250° [lit. (4) mp 246–247°].

Anal.—Calc. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.57. Found: C, 78.57; H, 10.61.

Betulinic Acid—Elution with petroleum ether–benzene (1:1) afforded 5.8 g of semicrystalline material, 4.1 g of which was decolorized and rechromatographed over silica gel. The latter gave mostly ursolic acid along with 0.25 g of another crystalline material. Recrystallization from methanol gave needles, mp 284–286°, which was undepressed upon admixture with an authentic specimen[5] of betulinic acid. The PMR, IR, and mass spectra of the two samples were identical.

Anal.—Calc. for $C_{30}H_{48}O_3 \cdot CH_3OH$: C, 76.18; H, 10.72. Found: C, 75.86; H, 10.67.

Uvaol—The first eluate of the alumina column with petroleum ether–benzene (1:1) gave 12.5 g of a solid residue. Crystallization from acetone and recrystallization from chloroform–ethanol afforded pure material, mp 224–225°, whose PMR, IR, and mass spectra were sug-

[1] Identification was confirmed by Dr. Robert E. Perdue, Medicinal Plant Resources Laboratory, Agricultural Research Center, Beltsville, Md. A reference specimen was deposited in that herbarium. The plant was collected in Coahuila, Mexico, in July 1970.

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[3] Carbon and hydrogen analyses were performed by Chemalytics, Inc., Tempe, Ariz. PMR, IR, and mass spectra were determined using a Varian T-60 spectrometer, a Beckman IR-33, and a Hitachi Perkin-Elmer double-focusing spectrometer (model RMU-6E), respectively. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

[4] From this laboratory.

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

gestive of uvaol [lit. (5) mp 222–224°]. The diacetate had a melting point of 151–152° [lit. (6) mp 150–151°]. An authentic specimen of uvaol, prepared by the lithium aluminum hydride reduction of methyl ursolate (7), had a melting point of 223–224°, which was undepressed upon admixture with the sample from *V. corymbosa*.

Anal.—Calc. for $C_{30}H_{50}O_2$: C, 81.38; H, 11.38. Found: C, 81.51; H, 11.67.

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Preparation of Tri- and Tetramethyleneisoxazoles

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Abstract □ The syntheses of several tri- and tetramethyleneisoxazoles resulting from the condensation of C(α)O-dilithioximes with esters, followed by acid cyclization, are described.

Keyphrases □ Isoxazoles, tri- and tetramethylene—synthesized by condensation of 1,4-dianions with esters followed by acid cyclization □ Tri- and tetramethyleneisoxazoles—synthesized by condensation of 1,4-dianions with esters followed by acid cyclization

The preparation of various trimethyleneisoxazoles and tetramethyleneisoxazoles was accomplished by the condensation of the 1,4-dianions of cyclopentanone and cyclohexanone oximes with several esters.

DISCUSSION

One major use of isoxazoles and their derivatives is in the preparation of various pharmacologically important agents. These isoxazoles are prepared by synthetic routes which give more than one isomeric product, and separation of these materials can be difficult. Until the initial report on the preparation of unsymmetrical 3,5-disubstituted isoxazoles from the 1,4-dianions of aromatic oximes containing an α-hydrogen atom (1), no fundamentally new synthetic routes appeared (2).

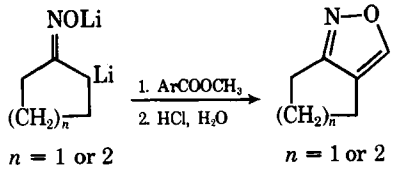
For example, the most widely applicable method for the preparation of the isoxazole ring system is by the reaction of hydroxylamine with a 1,3-dicarbonyl compound (3); since two bonds are being formed to close the heterocyclic ring, two isomers (A and B) are formed.

A: one bond formed

B: two bonds formed

This investigation focused on the preparation of tri- and tetramethyleneisoxazoles. The literature indicates that these materials can be obtained from the 1,3-dicarbonyl compounds, benzoylcyclopentanone and benzoylcyclohexanone, which are not common reagents and are difficult to prepare (4). In addition, two products are possible; they were isolated and characterized previously.

This report deals with the preparation of these isoxazoles by the condensation of the 1,4-dianions of cyclopentanone and cyclohexanone oximes with esters such as methyl nicotinate, methyl furoate, and methyl anisate (Scheme I). The precyclization intermediate was not isolated.



$n = 1$ or 2 $\qquad$ $n = 1$ or 2

Scheme I

All atoms of the heterocyclic ring are in position prior to cyclization, and the cyclization involves the formation of only one bond, which means only one isomer is formed and not a mixture of isomers. Table I gives the analytical and absorption spectral data for new material.

The syntheses described for the preparation of these isoxazoles have several advantages over other methods (2, 3, 5). The experimental procedure is efficient and requires the use of readily available starting materials; it is an unequivocal method for the preparation of a single isomer of unsymmetrically substituted isoxazoles.

EXPERIMENTAL

To a stirred solution of 0.025 mole of oxime in 100 ml of tetrahydrofuran, which was cooled to 0° under a nitrogen atmosphere, was added 0.05 mole of 1.6 *M* *n*-butyllithium[1] during 5 min. The mixture was stirred for 45 min and condensed with a 0.0125-mole sample of ester dissolved in 50–75 ml of tetrahydrofuran. After stirring for 15–30 min at 0°, the mixture was neutralized with 100 ml of 3 *N* hydrochloric acid.

The entire mixture was heated, with good stirring, under reflux for 1 hr and cooled; the phases separated. The aqueous layer was neutralized with sodium bicarbonate and extracted with three 75-ml portions of ether. The combined organic extracts were dried over

[1] Lithium Corporation of America, Bessemer City, N.C.