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STUDIES ON ZOAPATLE, VII. ANGELOYLGRANDIFLORIC ACID, A SPONTANEOUS UTERINE CONTRACTION INHIBITOR (SUCI) FROM *MONTANOA TOMENTOSA* SSP. *TOMENTOSA*[1]

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As part of our ongoing investigation of *Montanoa tomentosa* Cerv. ssp. *tomentosa* (Compositae), several fractions and isolates have been tested in guinea pigs for both in vitro activity [uterine stimulant (US^+), spontaneous uterine contraction inhibitory ($SUCI^+$)] and in vivo activity [uterine evacuation (UE^+)]. Kaur-9(11),16-dien-19-oic acid, isolated from the leaves of *M. tomentosa*, had previously been reported to be US^+ (1), which activity was confirmed in our laboratory (2,3). However, both this compound and its methyl ester derivative were shown to be UE^- (2,3). Zoapatanolide A and zoapatanol, on the other hand, were shown to be $SUCI^+$ and UE^+ (2,3), although a further compound, tomexanthin, was found to be $SUCI^+$ and UE^-. The present study was undertaken in an attempt to isolate further constituents which might have the desired in vivo (UE^+) activity and to determine how such activity might correlate with in vitro results; chemical modification was anticipated for isolates, as appropriate, prior to testing in vivo.

Isolated during the course of this study were the kaurene diterpenes, (−)-kaur-16-en-19-oic acid, (−)-kaur-9(11),16-dien-19-oic acid (grandiflorenic acid), monoginoic acid, grandifloric acid (grandiflorolic acid), and angeloylgrandifloric acid (angeloylgrandiflorolic acid), as well as the ubiquitous sterol stigmasterol. In addition, methyl kaur-16-en-19-oate was prepared by methylation of (−)-kaur-16-en-19-oic acid. (−)-Kaur-16-en-19-oic, kaur-9(11),16-dien-19-oic, and monoginoic acids had been found in this plant previously (4), whereas the occurrence of grandifloric and angeloylgrandifloric acids in this genus is being reported for the first time.

In vitro bioassay showed that angeloylgrandifloric acid inhibited the spontaneous contractions of guinea pig uterine strips ($SUCI^+$) at a concentration of 1.2 mg/ml, whereas (−)-kaur-16-en-19-oic and monoginoic acids were inactive ($SUCI^-$) at concentrations of 4.0 and 4.6 mg/ml. The desire to conserve material for in vivo evaluation precluded the testing of methyl kaur-16-en-19-oic acid in vitro at this time. In the in vivo, 22-day, pregnant guinea pig bioassay, methyl kaur-16-en-19-oic and angeloylgrandifloric acids were both found to be inactive (UE^-) at the dose of 100 mg/kg i.p. Previously, we had shown kaur-9(11),16-dien-19-oic acid and its methyl ester to be UE^- at doses of 100 and 200 mg/kg, respectively (2,3); we had tested only the former in vitro and had reported it to be US^+ at a concentration of 0.21 mg/ml. Paucity of material precluded in vivo testing at this time of (−)-kaur-16-en-19-oic acid and monoginoic acid and any testing of grandifloric acid.

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The fertility-regulating significance, if any, of the $SUCI^+$ activity of angeloylgrandifloric acid remains unclear at present. Although we had previously found two *M. tomentosa* fractions and two of the isolates therefrom to be $SUCI^+$ and UE^+ (2,3), we now have found a second isolate to be $SUCI^+$ but UE^- at the concentrations/doses tested. Consequently, whether $SUCI^+$ activity can be used as a predictor for in vivo fertility-regulating activity remains to be determined.

EXPERIMENTAL

PLANT MATERIAL.—The leaves and roots of *M. tomentosa* Cerv. ssp. *tomentosa* used in this investigation were harvested in the summers of 1982 and 1985, respectively, from plants cultivated at our Pharmacognosy Field Station, Lisle, Illinois. Herbarium specimens have been deposited at the Field Museum of Natural History, Chicago, IL.

EXTRACTION, FRACTIONATION, AND ISOLATION.—Ground leaves of *M. tomentosa* (24.5 kg) were extracted by percolation with EtOAc. Removal of the solvent afforded a residue (2.66 kg) which was defatted with hexane. Chromatography of a portion (2.1 kg) of this fraction led to the isolation of (−)-kaur-9(11),16-dien-19-oic acid (23.1 g) and angeloylgrandifloric acid (8.3 g). Ground roots (23 kg) of the plant were exhaustively extracted with hot MeOH. Removal of the solvent left a residue (1.55 kg) which was partitioned between EtOAc and H_2O (1:1) to give EtOAc (0.71 kg) and aqueous-soluble (0.84 kg) fractions. Following the harvesting of a precipitate (136.3 g), the mother liquor of the EtOAc fraction was chromatographed on Si gel. Subsequent work-up of the resulting fractions afforded (−)-kaur-16-en-19-oic (910 mg), monoginoic (5.9 g), angeloylgrandifloric (4.5 g), and grandifloric (110 mg) acids. The ubiquitous stigmasterol (1.4 g) was also obtained.

ISOLATE IDENTIFICATION.—Identification of the isolates was made by comparison of their physical and spectroscopic properties with published data (4-10).

PREPARATION OF METHYL KAUR-16-EN-19-OATE.—Methyl kaur-16-en-19-oate was prepared by methylation of (−)-kaur-16-en-19-oic acid with CH_2N_2 and subsequent work-up in the usual manner.

BIOASSAYS.—The in vitro assays (2,3) were conducted using estrogenized guinea pig uterine strips as described by Ponce-Monter *et al.* (11); isometric contractions of the uterine strips were recorded. Under these conditions in our laboratory, regular spontaneous uterine contractions can be observed to continue for several hours. It is the effect of test substances on these spontaneous contractions that is being reported. Test substances were solubilized as their polyvinylpyrrolidone (10,000) co-precipitates in a 1:4 ratio (12) and added to the tissue bath. Two types of effect were observed: an increase in the frequency and/or tone of muscle contractions (US^+) or a decrease in, or elimination of, the spontaneous uterine contractions ($SUCI^+$).

The in vivo assays (2,3) were conducted as described by Hahn *et al.* (13). Guinea pigs (Hartley) were obtained from CAMM Laboratory Animals, Wayne, NJ, at day 19 of gestation to allow acclimatization. On day 22, the test compounds and the vehicle[6] were administered i.p. (minimum of four guinea pigs per treatment group; one vehicle treatment group per two or three test compound treatment groups). Autopsies were performed on the fourth day post-dosing to evaluate the uterine contents; a significant decrease (ANOVA) in the number of viable fetuses was considered a positive response (UE^+).

Full details of compound isolation/preparation and identification are available from the authors on request.

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ACTIVE CRYSTALLINE PRINCIPLES FROM *HERACLEUM BRUNONIS*

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Naturally occurring coumarins form an important group of plant products with a wide range of physiological actions (1). Xanthotoxin, bergapten, imperatorin, and angelicin are among the best dermal photosensitizers used for the treatment of leucoderma (2). Imperatorin has been regarded as the best antifungal agent (3) among the coumarins, whereas xanthotoxin has been found to exhibit tuberculostatic activity (4). Xanthotoxin and bergapten have also been reported to show molluscicidal activity against *Biomphalaria biossi* (5).

The presence of these bioactive coumarins in *Heracleum brunonis* Benth. (Umbelliferae), in addition to other compounds as reported in our earlier (6,7) and present communication, bring this Himalayan plant to light as an active herb possessing antidermal, antifungal, tuberculostatic, and molluscicidal properties.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded with the following instruments: uv, Hitachi model 220; ir, Perkin-Elmer model 298; ^{1}H nmr, 80 MHz, Varian CFT-20; and ms, JEOL JMS-D 300, mass spectrometer.

PLANT MATERIAL.—The roots of *H. brunonis* were collected from glacier regions of Kumaon Himalaya, U.P., India, at an altitude of 4000 m in September 1984. The plant material, with voucher No. herb. FRI (DD) Lace 1673, is available in the herbarium of the Forest Research Institute, Dehradun, where the plant material was identified.

EXTRACTION AND ISOLATION.—The dried roots (700 g) of *H. brunonis* were powdered, extracted with 80% MeOH, concentrated under reduced pressure, and re-extracted with $CHCl_3$-H_2O (1:1). The $CHCl_3$ layer was separated, concentrated, and further extracted with petroleum ether (60-80°). The petroleum ether extract was chromatographed on a column of Si gel G and eluted with different proportions of petroleum ether, C_6H_6, and EtOAc. Apart from the furanocoumarins, bergapten, imperatorin, (+)-heraclenol, columbianadin and (+)-columbianetin reported in our earlier communication (6,7), four additional compounds have been isolated by repeated column chromatography, tlc, and reverse phase hplc methods. Three of them were fluorescent compounds (365 nm, uv light) that gave positive tests for coumarins (8), and one was an anthraquinone (9,10); the compounds have been identified (8,10-13) as xanthotoxin (120 mg), angelicin (90 mg), pimpinellin (70 mg), and chrysophenol (35 mg) by means of mp, mmp, uv, ir, ^{1}H nmr, ^{13}C nmr, and ms as well as by comparisons with authentic samples.

The absence of pyranocoumarins is also of chemotaxonomic significance in the genus *Heracleum* (8,14-16).