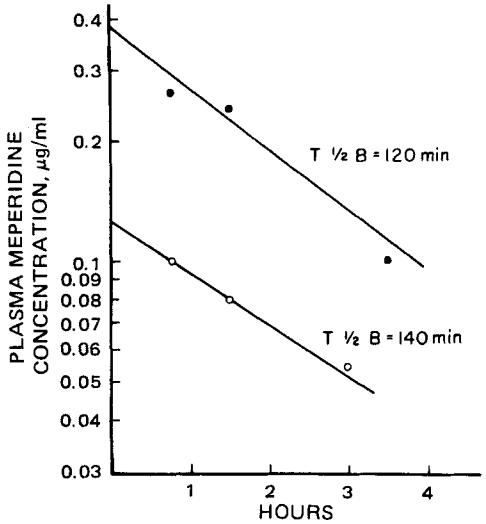

Figure 2—*Plasma concentration–time profile for meperidine in two infants following therapeutic drug administration. Sample size ranged from 0.5 to 1.5 ml of plasma.*

estimate and an intercept of zero. Extraction efficiency based on results from 10 samples using ^{14}C-meperidine hydrochloride was estimated at 85%.

Results of meperidine analysis in two infants following therapeutic intravenous administration of the drug are shown in Fig. 2. Although the calculated half-life was 1.5 hr for both infants, no clear definition of biological half-life in infants can be made since only two subjects were available for complete analysis. Burns *et al.* (1) demonstrated a biological half-life of 3–4 hr in adult humans with variations up to 100%.

This rapid method of analysis may prove useful for meperidine determinations when sample size is limited and many samples must be analyzed.

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Flavonoid Constituents from *Eupatorium altissimum* L. (Compositae)

R. H. DOBBERSTEIN, M. TIN-WA, H. H. S. FONG, F. A. CRANE, and N. R. FARNSWORTH [x]

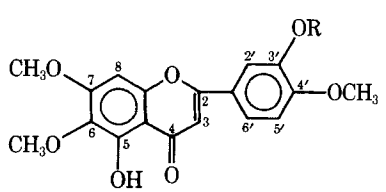
Abstract □ An aqueous ethanol extract of *Eupatorium altissimum* L. (Compositae) showed confirmed activity in the P-388 lymphocytic leukemia assay in mice, and the chloroform solubles showed both cytotoxic activity in the 9KB carcinoma of the nasopharynx cell culture assay and antitumor activity in the P-388 lymphocytic leukemia assay. Two flavones, eupatorin and 5-hydroxy-3′,4′,6,7-tetramethoxyflavone, were isolated and identified. Both were devoid of cytotoxic and antitumor activity.

Keyphrases □ *Eupatorium altissimum*—aqueous ethanol whole plant extract, two flavones isolated, cytotoxic and antitumor activity evaluated □ Flavones—isolated from *Eupatorium altissimum* aqueous ethanol whole plant extract, cytotoxic and antitumor activity evaluated □ Cytotoxic activity—evaluated in *Eupatorium altissimum* aqueous ethanol whole plant extract and isolated flavones □ Antitumor activity—evaluated in *Eupatorium altissimum* aqueous ethanol whole plant extract and isolated flavones

As part of a continuing search for antitumor compounds from plant sources, it was found that an aqueous ethanol extract of *Eupatorium altissimum* L. (Compositae) showed confirmed activity against the P-388 leukemia assay in mice and that the chloroform solubles showed both antitumor activity in the P-388 leukemia and marginal activity against the 9KB cell culture (cytotoxicity). In the only previous investigation of *E. altissimum*, a defatted ethanol extract was reported to exhibit antimicrobial activity against *Leuconostoc citrovorum* (1).

Extracts of various *Eupatorium* species have been reported to possess cytotoxic and/or antitumor activity. Cytotoxic flavonoids have been isolated from *E. semiserratum* (2–4) and *E. cuneifolium* (4); cytotoxic and antitumor sesquiterpene lactones have been isolated from *E. semiserratum* (5), *E. cuneifolium* (6, 7), *E. formosanum* (8), and *E. rotundifolium* (9–11).

In this investigation, two flavonoids were isolated from a fraction possessing cytotoxic activity. One was identified as eupatorin (I), a flavone previously isolated from *E. semiserratum* (2). The other flavonoid was verified as 5-hydroxy-3′,4′,6,7-tetramethoxyflavone (II). Although II



I: R = H

II: R = CH_3

was synthesized previously (12), it was only recently isolated from a natural source, *Salvia lavanduloides* (13). This paper reports the first isolation of these two flavones from *E. altissimum*.

EXPERIMENTAL[1]

Biological Activity—A 50% aqueous ethanol extract of *E. altissimum* was evaluated for cytotoxicity and antitumor activity[2]; it was active against the P-388 lymphocytic leukemia in mice[3] (T/C 144, 133, and 144 at 150, 100, and 66 mg/kg, respectively). The chloroform solubles, obtained by partitioning the concentrated aqueous ethanol extract between chloroform and water, possessed both cytotoxic[4] (ED_{50} = 20 μg/ml) and antitumor activity (T/C 150 and 159 at 200 and 100 mg/kg, respectively).

Extraction and Fractionation—A sample of whole plant material (5.5 kg) was continuously extracted[5] with petroleum ether (bp 30–60°) until the extract was colorless. The extract was then concentrated *in vacuo* to a viscous syrup weighing 296 g (Fraction A). The defatted plant material was air dried and then continuously extracted with methanol until the extract was nearly colorless. The methanol extract was concentrated *in vacuo* to yield 599 g of a thick dark-green syrup (Fraction B).

Fraction B was partitioned between chloroform (2 liters) and distilled water (2 liters). After two additional extractions of the aqueous layer with chloroform (2 liters), the combined chloroform fraction (Fraction C) was dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to give a residue weighing 172 g. The aqueous phase was lyophilized to yield a residue weighing 387 g (Fraction D). A precipitate, which formed between the chloroform and water phases, was redissolved in methanol and concentrated *in vacuo* to a viscous brown syrup weighing 35 g (Fraction E).

Fraction C was partitioned between petroleum ether (bp 30–60°; 2 liters) and 20% aqueous methanol (2 liters). The petroleum ether fraction (Fraction F) was separated and concentrated *in vacuo* to a viscous green syrup weighing 32 g. The aqueous methanol phase was filtered, and a dark-green waxy material weighing 19 g (Fraction G) was collected. The filtrate was concentrated *in vacuo* to remove the methanol, diluted with distilled water (500 ml), and lyophilized to yield Fraction H (118 g).

Chromatography of Fraction H—A portion (25 g) of Fraction H was dissolved in chloroform and carefully added to the top of a chloroform slurry-packed column containing 500 g of silica gel PF_{254}[6] (Column 1). Elution was initiated with chloroform, and 20-ml fractions were collected.

The eluent was changed by the addition of increasing amounts of methanol as the fractions were monitored by TLC[7]. The fractions were combined into 24 groups on the basis of their TLC patterns.

Isolation and Characterization of Eupatorin (I)—Fraction 8 (690 mg), which was eluted from the column with chloroform–methanol (99:1), was dissolved in benzene–ethyl acetate (6:1) and rechromatographed over a column of silica gel PF_{254}. Elution was initiated with benzene–ethyl acetate (6:1), and 20-ml fractions were collected. The polarity of the eluent was increased by the addition of ethyl acetate. Finally, the column was washed with methanol. Fractions were combined into 18 groups according to their similarity following TLC[8]. Yellow crystals (I, 460 mg) spontaneously crystallized from fractions 13–15. Recrystallization from benzene–chloroform gave 245 mg of yellow needles, mp 189–190°. Additionally, a small sample (20 mg) was recrystallized from dioxane–water, mp 191–192°.

A UV spectrum of I in methanol exhibited absorptions with λ_{max} at 342 (log ε 4.50), 274 (4.35), 254 (4.32), and 243 (4.34) nm. Bands were observed in the IR spectrum (KBr) at ν_{max} 3430 (OH) and 1658 (chelated CO) cm^{-1}. These data are typical of flavones having a hydroxyl substituent at C-5 and hydroxyl or methoxyl substituents at both C-3′ and C-4′ positions (15–17). The mass spectrum showed a molecular ion at *m/e* 344 (100%), in agreement with an empirical formula of $C_{18}H_{16}O_7$. The NMR spectrum of I in dimethyl sulfoxide-d_6 was in good agreement with that reported for eupatorin (4).

The TLC, UV, IR, and mass spectral properties of I were identical with those of authentic eupatorin[9], and there was no depression of the melting point following a mixed melting-point determination. This evidence establishes the identity of I as eupatorin.

Isolation and Characterization of II—Fraction 5 (860 mg), which had been eluted from Column 1 with chloroform, was dissolved in benzene–ethyl acetate (4:1) and rechromatographed over a column of silica gel PF_{254} using benzene–ethyl acetate (4:1) as the eluent. Fractions (20 ml) were collected and combined into 13 groups on the basis of their TLC[8] similarities. Fractions 6–9 were combined and evaporated to dryness *in vacuo* (210 mg). Crystallization and recrystallization of this material from 95% ethanol yielded pale-yellow microcrystals (II, 142 mg), mp 188–189°.

The UV spectrum of II in methanol showed absorption maxima at 339 (log ε 4.49), 275 (4.32), and 242 (4.34) nm; the IR spectrum (KBr) showed bands at ν_{max} 3410 (OH) and 1660 (chelated CO) cm^{-1}. Again these data suggested a flavone nucleus having a hydroxyl substituent at C-5 and hydroxyl or methoxyl substituents at both C-3′ and C-4′ positions. The mass spectrum of II showed a molecular ion at *m/e* 358 (100%, $C_{19}H_{18}O_7$) with significant ions at *m/e* 343 (94%), 329 (22), 315 (23), 312 (25), 181 (17), and 153 (40). The NMR spectrum of II in deuterochloroform showed a 3H singlet at δ 3.92 (OCH_3), a 9H singlet at δ 4.00 (3-OCH_3), a 1H singlet at δ 6.58 (olefinic H), a 1H singlet at δ 6.61 (ArH), a 1H doublet at δ 7.01 [J = 9 Hz (ArH)], a 1H doublet at δ 7.37 [J = 2 Hz (ArH)], a 1H doublet of doublets at δ 7.64 [J = 9 Hz, J = 2 Hz (ArH)], and a 1H singlet at δ 12.76 (ArOH).

These data suggested that II was eupatorin 3′-methyl ether. Therefore, a sample of eupatorin was methylated. Eupatorin (52 mg) in methanol (100 ml) was treated with excess diazomethane (18) in ether at room temperature for 24 hr. The solvent was removed *in vacuo*, and the product, dissolved in benzene–ethyl acetate (6:1), was chromatographed over a column of silica gel PF_{254} (5 g) using benzene–ethyl acetate (6:1) as the developing solvent. The second fraction eluted (25 mg) was crystallized from 95% ethanol to give pale-yellow microcrystals (20 mg), mp 188–189°. Comparison of the TLC, UV, IR, NMR, and mass spectral properties of methylated I with those of II showed that they were identical.

Finally, an authentic sample of II[9] was shown to be identical with the isolated product in all respects. There was no depression of the melting point following a mixed melting-point determination.

DISCUSSION

As part of a program of screening plants for antitumor activity, it was observed that an aqueous ethanol extract of *E. altissimum* (Compositae) showed confirmed *in vivo* activity against the P-388 lymphocytic leukemia assay in mice. The chloroform-soluble fraction was marginally active against Eagle's 9KB carcinoma of the nasopharnyx in cell culture (cytotoxic) and active against the P-388 lymphocytic leukemia assay in mice. Compounds I and II were isolated from Fraction C. Both compounds, however, were inactive in the two test systems.

Eupatorin (I) was previously isolated from *E. semiserratum* and was reported to show cytotoxic activity (2, 4). However, the cytotoxicity of I, isolated from *E. altissimum*, could not be confirmed even after repeated testing.

Flavone II was recently isolated from *S. lavanduloides* (Labiatae) (13), representing its first reported natural occurrence. The present investigation establishes the first occurrence of this compound in the genus *Eupatorium* of the unrelated Compositae family.

Work is currently in progress to isolate and identify the compound(s) present in *E. altissimum* responsible for the antitumor activity.

[1] The plant material consisted of the whole plant of *E. altissimum* L. (Compositae), collected in Lisle, Ill., during October 1972. Identification was made by Floyd A. Swink of the Morton Arboretum, Lisle, Ill. Voucher specimens are deposited in the Herbarium of the Department of Pharmacognosy and Pharmacology, University of Illinois at the Medical Center, Chicago, IL 60612.

Melting points were determined using a Kofler hot-stage instrument and are uncorrected. Mixed melting points were determined on a Thomas-Hoover Uni-Melt apparatus. UV spectra were recorded using a Beckman model DB-G grating spectrophotometer. IR spectra were run using a Beckman model IR-18A spectrophotometer. NMR spectra were recorded on a Varian T-60A instrument at 60 MHz. Mass spectral data were obtained on a Hitachi Perkin-Elmer model RMU-6D spectrophotometer.

[2] The test methods employed were those of the Drug Research and Development Branch of the National Cancer Institute (14).

[3] An active fraction is one that exhibits a T/C of ≧125%.

[4] An active fraction is one that exhibits an ED_{50} ≦20 μg/ml.

[5] Lloyd extractor.

[6] Silica gel PF_{254} for preparative layer chromatography, E. Merck, Darmstadt, Germany.

[7] TLC analyses were carried out on silica gel G precoated plates (E. Merck, Darmstadt, Germany) using chloroform–methanol (19:1) as the solvent. The developed plates were sprayed with 70% methanolic sulfuric acid and were heated at 100° for 5 min.

[8] TLC analyses were conducted on silica gel G precoated plates using benzene–ethyl acetate (2:1) as the developing solvent.

[9] Supplied by Professor L. Farkas, Hungarian Academy of Sciences, Budapest, Hungary, and/or by Professor N. Morita, University of Toyama, Japan.

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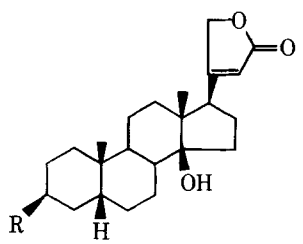
Thiocardenolides II: Synthesis and Pharmacological Evaluation of 3β-Thioacetyl-14β-hydroxy-5β-card-20(22)-enolide

HANLEY N. ABRAMSON [x], CHIAN L. HUANG, CHIU-CHUNG HO, LAWRENCE L. WALNY, and YOU-MEEI HSU

Abstract □ The synthesis of a 3β-thioacetylcardenolide is described. The thioacetate exhibited effects similar to those seen with digitoxigenin acetate on the isolated frog and guinea pig hearts at 1×10^{-7} dilution. In the intact rat heart, the lethal dose was 5 mg/kg for the thioacetate and 2.5 mg/kg for digitoxigenin acetate. The thioacetate inhibited sodium- and potassium-activated adenosine triphosphatase to the same extent as digitoxigenin, but it was somewhat less inhibitory than digitoxigenin acetate.

Keyphrases □ Thiocardenolides—3β-thioacetyl - 14β - hydroxy - 5β - card-20(22)-enolide synthesized, screened for cardiotonic activity and effect on sodium- and potassium-activated adenosine triphosphatase □ Cardiotonic activity—screened in 3β-thioacetyl-14β-hydroxy-5β-card-20(22)-enolide □ Adenosine triphosphatase, sodium and potassium activated—effect of 3β-thioacetyl-14β-hydroxy-5β-card-20(22)-enolide

The naturally occurring cardioactive steroids are widely used in the therapy of congestive heart failure and atrial arrhythmias and have low therapeutic indexes. There have been numerous efforts to synthesize and evaluate cardenolide derivatives with improved margins of safety, and the literature in this area was reviewed (1). As part of a program to replace oxygens of various cardenolides by sulfur atoms with the aim of separating the therapeutic and toxic actions of the digitalis glycosides, the synthesis and pharmacological actions of the 3β-thioacetyl analog (I) of digitoxigenin (II) are herein reported. A previous paper (2) described the preparation and biological actions of a 3β-thiocyanato analog.



I: R = $SCOCH_3$
II: R = OH
III: R = $OCOCH_3$

EXPERIMENTAL[1]

Chemistry—A solution of 584 mg (1.29 mmoles) of 3-epidigitoxigenin 3-methanesulfonate (2) and 577 mg (5.0 mmoles) of freshly recrystallized (ethanol–water) potassium thioacetate in 10 ml of dimethylformamide (freshly distilled over potassium hydroxide) was heated at 70–90° for 3 hr under dry nitrogen. The reaction mixture was poured onto ice, and the solid product was collected by filtration, dried, dissolved in chloroform,

[1] Melting points were taken on a Fisher-Johns melting-point stage and are uncorrected. UV absorption spectra were determined in 95% ethanol on a Beckman model DK2A recording spectrophotometer. IR absorption spectra were recorded in chloroform on a Beckman model 8 recording spectrophotometer. NMR spectra were determined on a Varian EM 360 spectrometer, using tetramethylsilane as the internal standard and deuterochloroform as the solvent. Microanalyses were conducted by Spang Microanalytical Laboratory, Ann Arbor, Mich. TLC was carried out using Merck silica gel G 254 (0.25-mm thick, analytical plates) or Merck silica gel PF 254 + 366 (0.75-mm thick, preparative plates). Analytical plates were visualized by charring with 5% $Ce(SO_4)_2$ in 6 *N* H_2SO_4; preparative plates were visualized under UV light.