

# Chemical Constituents of *Cannabis sativa* L. Root

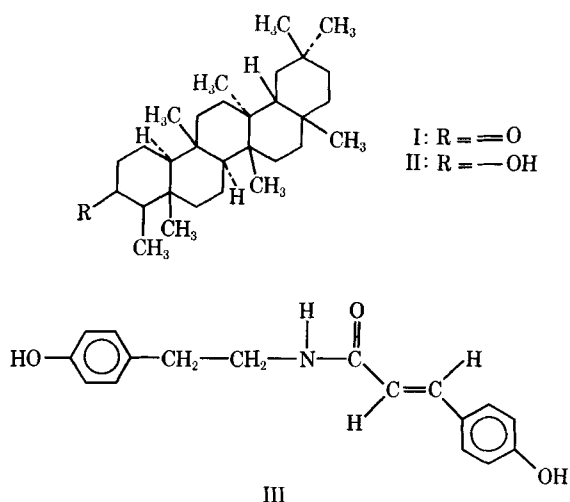
DAVID J. SLATKIN\*, NORMAN J. DOORENBOS, LOUIS S. HARRIS†, ASAAD N. MASOUD‡, MAYNARD W. QUIMBY, and PAUL L. SCHIFF, Jr.§

**Abstract** □ An extract of *Cannabis sativa* L. root yielded two pentacyclic triterpenes, friedelin and epifriedelanol, and *N*-(*p*-hydroxy- $\beta$ -phenylethyl)-*p*-hydroxy-*trans*-cinnamamide. Structures of the triterpenes were confirmed by preparation of derivatives and comparison to authentic friedelin. Epifriedelanol was also oxidized to friedelin. The amide was confirmed by synthesis.

**Keyphrases** □ *Cannabis sativa* L.—isolation and identification of chemical constituents □ Friedelin—isolated and identified from *Cannabis sativa* □ Epifriedelanol—isolated and identified from *Cannabis sativa* □ *N*-(*p*-Hydroxy- $\beta$ -phenylethyl)- $\beta$ -hydroxy-*trans*-cinnamamide—isolated and identified from *Cannabis sativa*

Marijuana grown under a National Institute of Mental Health contract afforded a large quantity of root material. An ethanolic extract of *Cannabis sativa* L. roots yielded the pentacyclic triterpene ketone, friedelin (friedelan-3-one) (I), its corresponding  $\beta$ -alcohol, epifriedelanol (II), and *N*-(*p*-hydroxy- $\beta$ -phenylethyl)-*p*-hydroxy-*trans*-cinnamamide (III). The triterpenes were characterized by preparation of derivatives and comparison with authentic friedelin<sup>1</sup>.

Friedelin was first isolated from cork as a mixture with cerin by Chevreul (1). In 1899, Istrati and Ostrogoich (2) separated this mixture and obtained the pure triterpene. The structure of friedelin, however, was not determined until 1956 by Corey and Ursprung (3). Friedelin and epifriedelanol have been isolated from numerous plant species, including *Salix japonica* (Salicaceae) (4), *Photinia glabra* (Rosaceae) (5), *Rhododendron* species (Ericaceae) (6), and *Ceratopetalum apetalum* (Cunoniaceae) (7).



<sup>1</sup> The reference samples were provided by Dr. Robert Stevenson, Department of Chemistry, Brandeis University, Waltham, Mass., and Dr. P. R. Jefferies and Dr. E. L. Ghisalberti, University of Western Australia, Nedlands, West Australia.

Table I—Mouse Tail-Flick Reaction Times<sup>a</sup>

Treatment	Premedication	20 Min.	60 Min.
Control, 50% dimethyl- sulfoxide	2.5	3.0	2.7
Amide:			
25 mg./kg. s.c.	2.2	3.1	2.8
50 mg./kg. s.c.	2.7	3.6	4.1
100 mg./kg. s.c.	2.7	6.4	4.8

<sup>a</sup> Six mice per dose. Values are in seconds.

The amide was characterized by synthesis. The only report of this compound was in 1968, when Rondest *et al.* (8) reported the isolation of this amide from the bark of *Evodia belahe* B. (Rutaceae). Analgesic activity of this amide was noted in a mouse behavioral test. To confirm this activity, the compound was run in a tail-flick test. The results are shown in Table I.

## EXPERIMENTAL<sup>2</sup>

**Plant Material**—Roots of *C. sativa* L. (Cannabinaceae) were used in this study<sup>3</sup>.

**Extraction**—Air-dried ground roots of *C. sativa* (4.7 kg.) were percolated with a total of 140 l. of ethanol. The extract was evaporated *in vacuo* at 40° to leave a brown syrup (155.6 g.). During the concentration, a white crystalline material (28 g.) precipitated. Recrystallization from benzene afforded white plates which, on TLC on silica gel G using a solvent system of chloroform-methanol (70:1) and visualization with a vanillin-sulfuric acid spray reagent (9), indicated the presence of two major spots, *R<sub>f</sub>* 0.73 and 0.61.

**Isolation of Triterpenes**—Chromatography of the crystalline triterpene mixture (500 mg.) on a column of neutral Woelm alumina, grade I (45 g.), gave a fraction eluted with 50% benzene in petroleum ether (30–60°) which yielded friedelin (150 mg.). Recrystallization of the triterpene from benzene gave white needles (60 mg.), m.p. 252.5–254.5° [lit. (6) m.p. 248–252°]; [ $\alpha$ ]<sub>D</sub><sup>20</sup> –23.9° (c 1.0, CHCl<sub>3</sub>) [lit. (6) [ $\alpha$ ]<sub>D</sub><sup>20</sup> –27.5° (c 0.34, CHCl<sub>3</sub>)]. An IR spectrum showed characteristic signals at  $\nu_{\max}$  1710 cm.<sup>–1</sup> (carbonyl stretch), 2925 and 2870 cm.<sup>–1</sup> (C—H stretch), and 1380 cm.<sup>–1</sup> (C—H bend). The molecular formula, as determined by high-resolution mass spectrometry, was found to be C<sub>30</sub>H<sub>50</sub>O, with a molecular ion of 426.3848 (calc. 426.3861). The fragmentations of friedelin were in accordance with those described by Courtney and Shannon (10). There was no depression of melting point when mixed with authentic friedelin, and IR spectra were superimposable. Friedelin oxime was prepared according to Stevenson (11) to give white plates, m.p. 289.5–292° [lit. (11) m.p. 289–292°]; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +29.0° (c 0.34, CHCl<sub>3</sub>). Friedelin-2,4-dinitrophenylhydrazone was prepared in the usual manner to give orange crystals, m.p. 292–294° dec. [lit. (6) m.p. 291° dec.]; [ $\alpha$ ]<sub>D</sub><sup>20</sup> +24.6° (c 0.33, CHCl<sub>3</sub>).

<sup>2</sup> Melting points were determined on a Thomas-Hoover Uni-melt melting-point apparatus and are corrected. IR spectra were run in KBr using a Perkin-Elmer 257. Optical rotations were determined on a Perkin-Elmer 141 polarimeter. Mass spectra were obtained by the Battelle Memorial Institute, Columbus, Ohio.

<sup>3</sup> Grown in the Marihuana Garden of the Department of Pharmacognosy, School of Pharmacy, University of Mississippi, during the summer of 1969. Voucher specimens are deposited in the Herbarium of the School of Pharmacy.

Further elution with additional 50% benzene in petroleum ether (30–60°) gave, on recrystallization from chloroform, white plates of epifriedelanol (100 mg.), m.p. 274.5–277° [lit. (4) m.p. 274°];  $[\alpha]_D^{25} + 18.9^\circ$  (c 1.0,  $\text{CHCl}_3$ ) [lit. (3)  $[\alpha]_D + 20.0^\circ$  (c 0.44,  $\text{CHCl}_3$ )]. An IR spectrum showed characteristic signals at  $\nu_{\text{max}}$  3490  $\text{cm}^{-1}$  (O—H stretch), 2925 and 2870  $\text{cm}^{-1}$  (C—H stretch), and 1365  $\text{cm}^{-1}$  (C—H bend). The molecular formula, by mass spectrometry, was found to be  $\text{C}_{30}\text{H}_{52}\text{O}$ , with a molecular ion of 428.4014 (calc. 428.4018). The acetate (prepared with pyridine and acetic anhydride) gave, on recrystallization from benzene-ethanol, white plates, m.p. 285–286° [lit. (4) m.p. 289°];  $[\alpha]_D^{25} + 26.0^\circ$  (c 0.25,  $\text{CHCl}_3$ ) [lit. (4)  $[\alpha]_D + 24.7^\circ$ ]. The benzoate (prepared with benzoyl chloride and pyridine) separated from ethanol in plates, m.p. 250–251° [lit. (3) m.p. 252–254°];  $[\alpha]_D^{25} + 31.2^\circ$  (c 0.25,  $\text{CHCl}_3$ ) [lit. (3)  $[\alpha]_D + 34.0^\circ$  (c 0.52,  $\text{CHCl}_3$ )]. Oxidation of epifriedelanol (20 mg.) with chromic acid in acetic acid, according to Fieser (12), yielded white needles of friedelin (12 mg.), m.p. 258–259°, identical with an authentic sample by comparison of a mixed melting point and IR spectra.

**Isolation of Amide**—The ethanolic residue was dissolved in ether (1.25 l.) and shaken with 1% hydrochloric acid ( $3 \times 1.25$  l.). The ether layer was dried over anhydrous sodium sulfate and evaporated to yield a residue (26.8 g., Fraction A). The acid extract was basified with concentrated ammonia solution and extracted with chloroform ( $3 \times 3.0$  l.). The chloroform solution was dried over anhydrous sodium sulfate and evaporated to give a residue (4.7 g., Fraction B).

Fraction B was chromatographed over silicic acid<sup>4</sup>-diatomaceous earth<sup>5</sup> (4:1) (173 g.). Elution with 1% methanol-chloroform and recrystallization from chloroform-methanol yielded white micro-rosettes of amide (7.5 mg.), m.p. 252–254° dec. [lit. (8) m.p. 252–255°]; UV  $\lambda_{\text{max}}^{\text{EtOH}}$  227 (log  $\epsilon$  4.1), 296 (log  $\epsilon$  4.1), and 312 nm. (log  $\epsilon$  4.1) [lit. (8) UV  $\lambda_{\text{max}}^{\text{EtOH}}$  226 (log  $\epsilon$  4.2), 288 (log  $\epsilon$  4.1), and 312 nm. (log  $\epsilon$  4.0)]; UV  $\lambda_{\text{max}}^{\text{EtOH}+\text{KOH}}$  243 (log  $\epsilon$  4.3), 312 (log  $\epsilon$  4.0), and 360 nm. (log  $\epsilon$  4.1) [lit. (8) UV  $\lambda_{\text{max}}^{\text{EtOH}+\text{KOH}}$  229 (log  $\epsilon$  4.4), 312 (log  $\epsilon$  4.0), and 351 nm. (log  $\epsilon$  4.0)]. The IR spectrum showed major absorptions of  $\nu_{\text{max}}$  3200 (broad, OH and NH stretch), 1650 (amide C=O stretch), 1590 and 1510 (aromatic C=C stretch), 1230 (phenolic C—O stretch), 980 (*trans*-disubstituted C=C bend), and 830  $\text{cm}^{-1}$  (*para*-substituted aromatic C—H bend). The molecular formula, by mass spectrometry, was found to be  $\text{C}_{17}\text{H}_{17}\text{NO}_2$ , with a molecular ion of 283.1233 (calc. 283.1208). The fragmentations were in exact agreement with those described by Rondet *et al.* (8). The synthesis of *N*-(*p*-hydroxy- $\beta$ -phenylethyl)- $\beta$ -hydroxy-*trans*-cinnamamide was carried out as described (8) from *p*-hydroxy-*trans*-cinnamic acid and tyramine in the presence of dicyclohexylcarbodiimide. The crystalline dicyclohexylurea was filtered from the reaction mixture, and the filtrate was chromatographed on silicic acid-diatomaceous

earth (4:1). Elution with 4% methanol-chloroform and recrystallization from hexane-acetone afforded synthetic *N*-(*p*-hydroxy- $\beta$ -phenylethyl)-*p*-hydroxy-*trans*-cinnamamide (618 mg.), m.p. 252–254° dec. The IR and UV spectra were superimposable with that of the isolate, and there was no depression of mixed melting point. TLC suggested that this amide is also present in Fraction A, the fraction in which one would have expected to find this substance because of its physical properties.

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\* American Foundation for Pharmaceutical Education—Edwin Leigh Newcomb Memorial Fellow.

† Present address: Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill, NC 27514

‡ Present address: School of Pharmacy, Creighton University, Omaha, NB 68131

§ Present address: Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15213

<sup>4</sup> Mallinckrodt.

<sup>5</sup> Celite, Johns-Manville Co.