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Keyphrases

Osmotic properties—aqueous solutions
Caffeine complexes
Complexing numbers—caffeine
Osmotic coefficient—calculated, equation
Thermoelectric osmometer—improved

Triterpenic Constituents of *Lepechinia chamaedryoides*

By MARIO SILVA

Betulinic acid was isolated and identified from *Lepechinia chamaedryoides* collected in the summer, and ursolic acid was found in plant material collected in the spring.

During a current project dealing with Chilean flora (1) the author had occasion to reexamine *Lepechinia chamaedryoides* in order to study the compound isolated by Alvarez (2), and to see how constant it is in this plant during the year.

The petroleum ether-soluble fraction of plant material collected during March (summer) yielded betulinic acid (3) characterized through its derivatives. Betulinic acid was also isolated from the benzene-soluble fraction. In this plant material no other triterpenic acid could be detected.

From plant material collected in November (spring), ursolic acid (4) was obtained which gave a methyl ester with a rather peculiar melting point (105°). Therefore, it was characterized through several derivatives. Betulinic acid could be detected by thin-layer chromatography only as its methyl ester.

These different results appear to be a seasonal variation of the triterpenic constituents.

EXPERIMENTAL[1]

Isolation of Betulinic Acid—Stems and leaves of *Lepechinia chamaedryoides* collected in March near Concepción, were dried at 80° with air circulation, and 2100 Gm. of the dried and ground plant was extracted in a Soxhlet apparatus with petroleum ether (b.p. 65–75°) to exhaustion. This solution was concentrated to yield 109 Gm. of a dark green product. This product was crystallized several times from ethanol to yield 11 Gm. of a crude acid m.p. 286°.

Betulinic Acid Methyl Ester—This ester was prepared by diazomethane treatment in ether solution; crystallized from ethanol as colorless crystals with m.p. 221°, $[\alpha]_D$ +4.4° (chloroform, c 0.44), $\nu_{max.}^{Nujol}$ 3610, 1695, and 1637 cm.$^{-1}$, $\nu_{max.}^{CHCl_3}$ 1724, 1647 cm.$^{-1}$.

Anal.—Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 79.34; H, 10.69.

Betulin Diacetate—The betulinic acid methyl ester on $LiAlH_4$ reduction using ether as solvent gave an noncarbonilic compound which on pyridine–acetic anhydride acetylation at room temperature gave a diacetate (5), m.p. 222°, $[\alpha]_D$ + 28.7° (chloroform, c 0.48), $\nu_{max.}^{Nujol}$ 1732, 1640, and 1250 cm.$^{-1}$, τ = 7.9 (two acetates).

Anal.—Calcd. for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33. Found: C, 77.73; H, 10.53.

The betulinic acid obtained was further characterized through several derivatives.

Benzene Extract—The defatted plant material was dried and the benzene-soluble constituents were extracted. This solution was concentrated to yield 27 Gm. of product. This product through crystallizations, first from petroleum ether and later with ethanol, gave in a good yield an acid, m.p. 285–286° identical with the betulinic acid previously isolated.

Isolation of Ursolic Acid—Stems and leaves of *Lepechinia chamaedryoides*, collected in November (spring) near Concepción, were dried at 80° with air circulation, and 4611 Gm. of the dried and ground plant was extracted in a Soxhlet apparatus with benzene to exhaustion. This solution was concentrated to yield 253 Gm. of a dark green product, and 150 Gm. of this product was chromatographed over alumina grade III to give crude ursolic acid m.p. 250°, $[\alpha]_D$ + 52.8° (pyridine, c 0.35).

Ursolic Acid Acetate—Treatment of the acid with pyridine–acetic anhydride gave an acetate, m.p. 248°,

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[1] Melting points (uncorrected) were performed on a Kofler block. Rotations were measured at 20°. The microanalyses were performed by Dr. Alfred Bernhardt's Institute, Mülheim, Germany, and at the Chemistry Department, Imperial College, London, England. Ultraviolet spectra were recorded in solution in absolute ethanol on a SP 700 spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 137 spectrophotometer. Nuclear magnetic resonance spectrum was determined on a Varian A-60 spectrometer using deuterochloroform as solvent and tetramethylsilane as an internal reference.

$[\alpha]_D$ + 77.5° (chloroform, c 0.40), $\nu_{max.}^{chloroform}$ 3165 (broad), 1730 and 1266 cm.$^{-1}$.

Anal.—Calcd. for $C_{32}H_{50}O_4$: C, 77.06; H, 10.11. Found: C, 77.36; H, 10.40.

The ursolic acid obtained was further characterized through several derivatives which were found to be identical with authentic samples.

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Keyphrases

Triterpenic constituents—*Lepechinia chamaedryoides*
Betulinic acid—summer plant growth
Ursolic acid—spring plant growth
TLC—identity
Optical rotation—identity
NMR spectrometry—identity
IR spectrophotometry—structure
UV spectrophotometry—structure

Coumarins VII. The Coumarins of *Lomatium nuttallii*

By KUO-HSIUNG LEE and T. O. SOINE

The ether extract of the root of *Lomatium nuttallii* (A. Gray) Macbr. has been examined and has provided a number of known coumarins. These are osthol, jatamansin, pteryxin, and lomatin. The ether extract also contained a new coumarin, provisionally named *nuttallin*, which has been identified as the senecioate ester of lomatin. The methanol extract yielded the glycosidic coumarin, columbianin.

INTEREST in naturally occurring agents with potential physiological activity has led the authors, in the past, to consider the *Lomatium* genus. At least one representative of this genus has been employed extensively by the American Indians as a therapeutic agent (1) without, however, any firm rationale for such usage. Our previous examinations have encompassed *L. columbianum* Math. and Const. (2). *L. suksdorfii* (Wats.) Coulter and Rose (3), and *L. dissectum* var. *multifidum* (Nutt.) Math. and Const. (4) and it has been determined that coumarins are the principal chemical type present. That coumarins can be responsible for a variety of physiological activities is well known (5). A continuing investigation of this genus has led the authors to examine the roots of *L. nuttallii* (A. Gray) Macbr. and has resulted in the isolation of a number of known coumarins. Specifically, osthol (I), lomatin angelate (II) [*i.e.*, jatamansin (6) or selinidin (7)] pteryxin (III), lomatin (IV),[1] and columbianin (V) (2, 8) have been isolated and identified. The presence of a new coumarin, lomatin senecioate (VI), provisionally named *nuttallin*, has been demonstrated. Naturally occurring nuttallin has proved to be elusive as far as isolation is concerned but its synthesis has been easily achieved following its identification in the NMR spectrum of the jatamansin residues. The fact that the spectrum of the mixture corresponded exactly for jatamansin (*i.e.*, lomatin angelate) was expected and it was not surprising that the peaks of any contaminant as closely related as a senecioate to an angelate of the same alcohol would coincide except for the specific protonic differences in the acid portion of the ester. These differences were observed as the singlets at τ 8.13 (3H), 7.84 (3H), and 4.34 (1H) which are the typical signals for the senecioyl group (9). The synthetic senecioate of the optically active natural lomatin had the NMR spectrum predicted from the previous observations on the crude mixture, *i.e.*, it peaks at τ 8.64 (6H, s.), 8.12 (3H, s.), 7.85 (3H, s.), 4.37 (1H, s.), 6.90 (2H, t.), 4.87 (1H, t.), 3.83 (1H, d.), 3.25 (1H, d.), 2.77 (1H, d.), and 2.42 (1H, d.). With the exception of the peaks noted above the spectrum is virtually identical with that of jatamansin. Curiously, nuttallin does not crystallize, a circumstance that is analogous to a similar situation with regard to the angelate (VII) and senecioate (VIII) esters of (−)-3′-hydroxy-3′,4′-dihydroxanthyletin. In this case, however, Lemmich *et al.* (10), in spite of extensive purification, were unable to secure VII in a crystalline form whereas Hata and Sano (9) had little difficulty in obtaining crystalline VIII (*i.e.*, decursin). The reasons for such anomalous behavior are not apparent.

EXPERIMENTAL

Melting points were determined in capillary tubes in a Thomas-Hoover melting point apparatus,

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[1] Reported in a preliminary communication: *J. Pharm. Sci.*, **53**, 990(1964).