$[\alpha]_D$ + 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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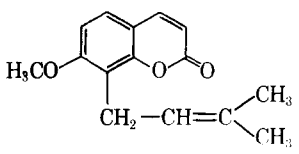

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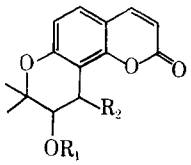
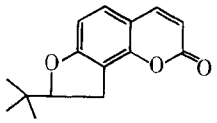
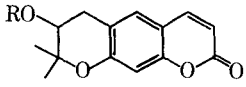


II, R_1 = angeloyl; R_2 = H
III, R_1 = acetyl; R_2 = angeloyloxy
IV, R_1 = H; R_2 = H
VI, R_1 = senecioyl; R_2 = H



O-β-D-gentiobiosyl



VII, R = angeloyl
VIII, R = senecioyl

checked for accuracy against a set of standard samples and are uncorrected.

Ultraviolet spectra were determined on a Bausch and Lomb Spectronic 505 recording spectrophotometer.

Infrared spectra were determined on a Perkin-Elmer 237B grating infrared spectrophotometer.

Values of [α] were determined on a Perkin-Elmer 141 polarimeter.

Nuclear magnetic resonance (NMR) spectra were determined on a Varian Associates A-60 instrument in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard. The authors are indebted to the School of Chemistry, University of Minnesota, for these determinations.

Silica gel for column chromatography refers to Baker Analyst No. 3405 activated at 120° and silica gel for thin-layer chromatography refers to Silica Gel G supplied by Brinkmann Instruments, Inc., Great Neck, N. J. activated at the same temperature.

Material[2]—*Lomatium nuttallii* (A. Gray) Macbr. is a species belonging to the *Umbelliferae* family of plants. It is known by the following pseudonyms: *Seseli nuttallii* (Gray), *Peucedanum graveolens* (Wats.), *P. kingii* (Wats.), *Cynamarathrum nuttallii* (Gray), and *Cogswellia nuttallii* (Gray) Jones. The present investigation was confined entirely to the roots of the plant which had been collected and identified in the summer of 1956 at an elevation of 8800 ft. in St. Charles Canyon, Bear Lake County, Idaho. The roots, which had been stored in a deep-freeze refrigerator, were reduced to a powder suitable for extraction in a Jacobsen laboratory grinder designed to keep frictional heat at a minimum.

Ether Extract—The dried and ground root (500 Gm.) was extracted with diethyl ether for several hours until only a faint fluorescence was evident in the menstruum when spotted on filter paper and examined under ultraviolet light. The marc was dried and reserved for methanol extraction (*q.v.*). Upon removal of the ethereal solvent a viscous oil remained. This oil was dissolved in 90% methanol, defatted with petroleum ether, and evaporated to give a residue weighing 30 Gm. A portion (15 Gm.) of this residue was chromatographed on silica gel (450 Gm.) impregnated with 10% of water. The column was successively eluted with benzene, benzene–chloroform, chloroform, chloroform–ethyl acetate, ethyl acetate, and ethyl acetate–methanol. Nine hundred and forty eight 20-ml. fractions were collected and the composition of the fractions determined by examining thin-layer chromatograms under ultraviolet light. The first benzene eluate (fractions 1–25) yielded only nonfluorescent, yellow-colored oily substances and was not investigated further. The subsequent fractions (26–184) contained a single spot having a purple-blue fluorescence under ultraviolet light and fractions 185–239 (0.6 Gm.) appeared to be a mixture of fluorescent compounds.

The benzene–chloroform mixture eluted the following fractions all of which contained a mixture of fluorescent compounds, *i.e.*, fractions 240–486, 487–498, 499–512, 513–524, 525–537, and 538–572.

Chloroform was used to elute fractions 573–763 which also appeared to be a mixture of fluorescent compounds.

The chloroform–ethyl acetate mixture eluted only traces of fluorescent substances in fractions 764–779 and 780–787. The same was true of the ethyl acetate and ethyl acetate–methanol fractions (788–948) and these fractions were not investigated further.

Isolation of Coumarins from the Ether Extract—The fractions containing fluorescent materials were worked up to yield the following:

(*a*) *Osthol*—The residue (4.1 Gm.) resulting from the removal of solvent from fractions 28–184 was recrystallized from a small amount of ether to provide fine, colorless needles, m.p. 81–82°; infrared spectrum (KBr disk): 3000, 2950, 2820 (triplet of aromatic OCH_3), 1721 (α-pyrone C=O), 1606, 1498, 1460 (aromatic C=C), 1563 (aromatic ring with conjugated C=C), 1272, 1245, 1120, 1085 (asymmetric and symmetric stretching of =C—O—C), and 820 cm.$^{-1}$ (1,2,3,4-aromatic substitution). The melting point of this compound was undepressed on admixture with an authentic sample of osthol and their infrared spectra were identical.

(*b*) *Jatamansin*—a blue-fluorescent oily substance (4.8 Gm.) was obtained from fractions 240–486. Thin-layer chromatography on silica gel using different solvent systems gave only one spot with the

[2] Collected and initially identified by Dr. T. G. Call, California State Polytechnic College, San Luis Obispo, California, with confirmation of the identification by Dr. Lincoln Constance, Botany Department, University of California, Berkeley. A voucher specimen has been placed in the herbarium of the Botany Department, University of Minnesota, Minneapolis.

same R_f value as that of jatamansin.[3] The oily substance (3.0 Gm.) was dissolved in a small amount of benzene and, after adding a few drops of cyclohexane, was kept in the refrigerator for 1 week. The white, crystalline semisolid that separated was removed by filtration and washed with cyclohexane to yield white crystals (83 mg.), m.p. 85–93°. Recrystallization from ether–cyclohexane provided colorless prisms (61 mg.), m.p. 90–92°; $[\alpha]_D^{25}$ −17.8° (c 1.0, $CHCl_3$); infrared spectrum (Nujol mull): 1724 (α-pyrone C=O), 1610, 1490 (aromatic C=C), 1245, 1147, 1119 (asymmetric and symmetric stretching of =C—O—C), and 847 cm.$^{-1}$ (1,2,3,4-aromatic substitution). It failed to depress the melting point of an authentic sample of jatamansin on admixture and the infrared spectra were identical.

After the isolation of jatamansin, the mother liquor was rechromatographed on silica gel impregnated with 3% of water using benzene, benzene–chloroform, and chloroform–ethyl acetate as eluants, successively. No further separations were realized and, similarly, thin-layer chromatography on silica gel failed to show any spots other than the one ascribable to jatamansin. The oily residue had an infrared spectrum quite similar to that of jatamansin but differed in minor details. The NMR spectrum agreed with that of jatamansin (6) but, in addition, had superimposed on it singlets at τ 8.13 (3H), 7.84 (3H), and 4.34 (1H). Further treatment of this fraction is deferred to a later portion of this paper (*q.v.*).

(*c*) *Pteryxin*—The orange-colored oily substance (1.66 Gm.) obtained from fractions 499–512 was rechromatographed on silica gel (50 Gm.) impregnated with 5% of water. Elution with chloroform furnished a colorless oily residue which was seeded with one small crystal of pteryxin[4] and the whole then kept in a refrigerator for 5 days. The colorless, large tetragonal crystals which formed were collected and twice recrystallized from skellysolve-B to give 21 mg. of white crystals, m.p. 81–83° undepressed by admixture with pteryxin; $[\alpha]_D^{21}$ −1.8° (c 0.69, $CHCl_3$): infrared spectrum (KBr disk): 1745 (α-pyrone C=O), 1615, 1495 (aromatic C=C), 1235, 1145 (asymmetric and symmetric stretching of =C—O—C), and 837 cm.$^{-1}$ (1,2,3,4-aromatic substitution). The infrared spectrum of this compound was identical to that of pteryxin.

(*d*) *Lomatin*—The reddish-orange oil obtained from the chloroform fractions (573–763) weighed 0.89 Gm. and was rechromatographed on silica gel (40 Gm.) impregnated with 5% of water. Elution with chloroform yielded a fraction having a single spot with the same R_f value as lomatin on examination by silica gel thin-layer chromatography. This fraction was dissolved in acetone and brought to turbidity with skellysolve-B at which time it was seeded with one small crystal of lomatin and kept in the refrigerator for 3 days. The resulting crystalline deposit was filtered and washed with benzene. Recrystallization from skellysolve-B–acetone mixture yielded colorless crystals (11.4 mg.), m.p. 179–181°; $[\alpha]_D^{28.5}$ +46.4 (c 0.44, C_2H_5OH). A mixed melting point with authentic lomatin showed no depression and the infrared spectra were identical.

Methanol Extract—The marc from the ether extraction was dried in air and extracted with methanol until a portion of the extract failed to give an appreciable residue when evaporated to dryness (7 days). This extract was allowed to concentrate spontaneously and deposited crystalline material as the volume decreased. The crystalline material was removed by filtration, rinsed with methanol, and dried, m.p. 270–275°. The filtrate and washings from these crystals was allowed to dry completely resulting in a thick reddish-brown syrupy residue. Paper chromatographic examination of this residue showed only one blue-fluorescent spot corresponding to that for the crystalline material. It was not examined further.

The crystalline material was recrystallized from hot methanol, m.p. 274–275° and showed no depression of melting point on admixture with authentic columbianin.[5] Similarly, an acetate prepared in the conventional manner, m.p. 220–222° failed to depress the melting point of authentic columbianin acetate.[5]

Hydrolysis of the Jatamansin Mother Liquors—The mother liquor, after removal of the crystalline jatamansin, was evaporated to yield a viscous, oily residue. This residue (360 mg.) was dissolved in 3 ml. of dioxane and 11 ml. of 1 *N* potassium hydroxide and left overnight at room temperature. Examination of the mixture by thin-layer chromatography showed that the faster moving fluorescent ester spot had disappeared almost completely in favor of a much slower moving fluorescent alcohol spot. The reaction mixture was diluted with an equal amount of water and acidified with 10% sulfuric acid. After standing for 45 min. it was adjusted to pH 8 with 10% sodium carbonate solution and finally extracted with chloroform. The chloroform extract, after drying and evaporation, was dissolved in benzene and chromatographed on silica gel (10 Gm.) impregnated with 5% of water. Initial elution with a mixture of chloroform–benzene (1:9) yielded a colorless, viscous oily substance (25 mg.) which had an infrared spectrum virtually identical with the starting material. Subsequent elution with chloroform afforded a crystalline material which upon recrystallization from acetone–skellysolve-B formed colorless needles (140 mg.), m.p. 180–181°; infrared spectrum (KBr disk): 3470 (free OH), 1700 (C=O), 1600, and 1484 (aromatic C=C), 1281 and 1075 cm.$^{-1}$ (OH deformation and C—O stretching). This compound showed no depression in melting point on admixture with authentic lomatin and the infrared spectra were identical. No other fluorescent materials were eluted by the use of ethyl acetate.

The aqueous phase (pH 8) was acidified with 10% sulfuric acid and extracted with ether. After drying and evaporation, the oily residue (*ca.* 110 mg.) was treated with dicyclohexylethylamine (250 mg.) and converted to the *p*-phenylphenacyl ester form according to the method of Stodola (11). The resulting white solid product (130 mg.) was then chromatographed on silica gel (10 Gm.) impregnated with 10% of water. The eluant was benzene–skellysolve-B (2:1) to which increasing amounts

[3] Kindly supplied by Dr. S. C. Bhattacharyya, National Chemical Laboratory, Poona, India.

[4] Obtained from the studies reported in *Reference 3*.

[5] Obtained in these laboratories from the studies reported in *Reference 2*.

of benzene were added. Unreacted *p*-phenylphenacyl bromide, a mixture of *p*-phenylphenacyl angelate and senecioate and *p*-phenylphenacyl senecioate (31 mg.), m.p. 136°, were obtained successively. The identity of the senecioate ester was established by thin-layer chromatography, infrared spectroscopy, and mixed melting point determination with an authentic sample of *p*-phenylphenacyl senecioate.

Synthesis of Lomatin Senecioate—Lomatin (200 mg.) was suspended in dry benzene (5 ml.). The suspension was added slowly to a solution of senecioyl chloride (b.p. 67°/39 mm. Hg) (230 mg.) in dry benzene (3 ml.). The mixture, protected by a calcium chloride drying tube, was refluxed on a steam bath for 16 hr. The reaction mixture was then cooled and washed with 5% sodium bicarbonate solution followed by distilled water. The benzene solution was dried over anhydrous magnesium sulfate, filtered, and the solvent removed under reduced pressure to yield a colorless, glassy substance (230 mg.). All attempts to crystallize the ester were unsuccessful. Thin-layer chromatography, however, showed only one spot with the same R_f value as noted for jatamansin and fractions 240–486. This compound was then further purified by silica gel chromatography and dried in a high vacuum to yield an amorphous, very hygroscopic substance with m.p. 58 ~ 62° $[\alpha]_D^{20}$ +75.0 (c 1.0, $CHCl_3$); ultraviolet spectrum: $\lambda_{max.}$ 326, 256, 246, and 219 mμ (log $\epsilon_{max.}$ 4.16, 3.73, 3.83, and 4.53, respectively); infrared spectrum (in $CHCl_3$) was almost identical with fractions 240–486, *i.e.*: 1724 (α-pyrone C=O), 1610, 1490 (aromatic C=C), 1140, 1115 (asymmetric and symmetric stretching of =C—O—C), and 834 cm.$^{-1}$ (1,2,3,4-aromatic substitution).

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Keyphrases

Coumarins—*Lomatium nuttallii*
Lomatium nuttallii—coumarins, isolated, identified
Nuttallin—new coumarin, isolated
Column chromatography—separation
TLC—identity
UV spectrophotometry—structure
IR spectrophotometry—structure
Optical rotation—identity
NMR spectrometry—identity

Gas-Liquid Chromatographic Determination of Dienestrol in the Presence of Methyltestosterone

By FRED L. FRICKE, STEPHEN M. WALTERS, and WILLIAM T. LAMPKIN

A gas chromatographic procedure has been developed for the determination of dienestrol in the presence of methyltestosterone. Dienestrol was extracted from tablets and quantitatively determined by gas chromatography of its bis-(trimethylsilyl)ether. The bis-(trimethylsilyl)ether of alizarin was used as an internal standard. Preliminary experiments indicate that the method is applicable in the presence of other androgens and corticosteroids.

THIS STUDY describes a rapid, specific, gas-liquid chromatographic procedure for the determination of dienestrol in the form of its bis(trimethylsilyl)ether derivative, with bis-(trimethylsilyl)acetamide as the silylating agent.

A number of analytical procedures have been reported for the determination of dienestrol. The USP XVI utilizes the Folin-Denis reagent in a colorimetric procedure. Cocking (1) also developed a colorimetric method based on a reaction of dienestrol with bromine in acetic acid, whereas Malpress (2) formed a polynitro derivative and measured the orange color produced. A method for the determination of dienestrol in biological samples, utilizing the formation and subsequent titration of the maleic acid adduct, was proposed by Smith (3). Gottlieb (4) formed nitrosophenols from various synthetic estrogens including dienestrol and measured the colors produced, while Gry (5) determined the nitroso derivative of dienestrol polarographically. Summa and Graham (6) determined dienestrol both by a polarograpic method, which is the basis of the NF XII assay, and by measurement of a yellow color [first observed by Banes (7)] that is formed with ultraviolet irradiation of dienestrol. In applications of gas chromatography as the determina-

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