Isolation and Identification of β-Amyrin—The semicrystalline precipitate from petroleum ether was crystallized from methanol to yield 0.592 g. of colorless needles, m.p. 197–199° [lit. (1) m.p. 194–200°]. The UV spectrum showed only end-absorption at λ_{max}^{MeOH} 206 nm. (log ε 3.48) and $[\alpha]_D^{25}$ + 89.4° (0.5, $CHCl_3$) [lit. (1) $[\alpha]_D^{25}$ + 87°]. An IR spectrum (KBr) was superimposable with that of an authentic sample of β-amyrin. An acetate derivative was prepared in the usual manner and exhibited m.p. 237–238° [lit. (1) m.p. 237–242°]. The identity of the isolate as β-amyrin was confirmed by a direct comparison with an authentic sample (mixed melting point, TLC, and IR).

Isolation and Identification of Ellagic Acid—On standing, a yellow compound (180 mg.) precipitated from the ethyl acetate fraction. This compound was insoluble in all common organic solvents; it was slightly soluble in hot pyridine but would not crystallize from this solvent. It did not melt below 350°, and it gave a green color with ferric chloride (phenolic). The compound was soluble in alkaline solution, giving a yellow color after the addition of alkali. It gave a positive Greissmayer test, indicating the presence of the 4,4′-dihydroxydiphenyl moiety (2). The UV spectrum of an impure sample showed absorption bands at λ_{max}^{MeOH} 366 and 255 nm. In the presence of sodium acetate, a new band appeared at 280 nm., with a corresponding weakening of the band at 255 nm. These UV data are in agreement with those published for ellagic acid (2). The IR spectrum (KBr) showed absorption bands at 3520 (OH), 1720 (unsaturated lactone), and 1601 (aromatic character) cm.$^{-1}$. An NMR spectrum in dimethyl sulfoxide showed only two aromatic protons in symmetric position at δ 7.5. A comparison by paper chromatography showed the isolate and a reference sample of ellagic acid to be identical.

A 30-mg. sample of the impure ellagic acid was heated under reflux with an excess of acetic anhydride for 15 hr. The resulting product was crystallized from dioxane to obtain colorless crystals, which began to sinter at 331° and melted at 338–341° [lit. (3) m.p. 343–346°]. A second 30-mg. sample was methylated in the usual manner with diazomethane. The product was crystallized from dioxane to yield pale-yellow crystals, m.p. 338–340° [lit. (4) m.p. 342–344°]. The IR spectrum (KBr) corresponded with published data for the tetramethyl ether of ellagic acid (4).

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* Present address: Institut für Pharmazeutische Arzneimittellehre der Universität München, Munich, Germany.

† Present address: Bergstrom Toxicology Laboratory, Rockville, MD 20851

▲ To whom inquiries should be directed.

Isolation of Daphnetin-8-β-glucoside from *Daphne papyracea*

RITEN NASIPURI and EGIL RAMSTAD▲

Abstract □ Daphnetin occurs free and bound as β-glucoside in the roots of *Daphne papyracea* Wall. ex Steud. The bound form, however, is not in the form of daphnin, as found in several Thymelaceae, but in the form of daphnetin-8-β-glucoside.

Keyphrases □ Daphnetin-8-β-glucoside—isolated from *Daphne papyracea* □ *Daphne papyracea*—isolation of daphnetin-8-β-glucoside

The isolation of three flavone glycosides was reported by Basu and Nasipuri (1) in their preliminary investigation of the roots of *Daphne papyracea* Wall. ex Steud., collected from Ranikshet, India. Later, Sharma *et al.* (2) reported the isolation of daphnin from the roots of the same plant. The present authors found that daphnetin occurs free and bound as 8-β-glucoside in the roots of the same plant[1] collected in Nepal.

[1] The plant was identified by Mr. P. Joshi, Pharmaceutical Expert to the Government of U.P., India, and the late Prof. N. K. Basu of Banaras Hindu University, Banaras, India. A specimen of the plant was deposited in the Department of Pharmaceutics, Banaras Hindu University, Banaras, India.

DISCUSSION

Two crystalline compounds were isolated: A, m.p. 255–256° dec.; and B, m.p. 229–230°, $[\alpha]_D^{25}$ −92° (solvent: 50% alcohol).

Acid hydrolysis of Compound B provided A_1, identical to Compound A, as shown by its chromatographic behavior, no depression of melting point by admixture with Compound A, and identical UV and IR spectra. The hydrolysate of B contains glucose, identified by chromatography and chromogenic tests. Emulsin hydrolyzed B into A and glucose, showing B to be a β-glucoside of A.

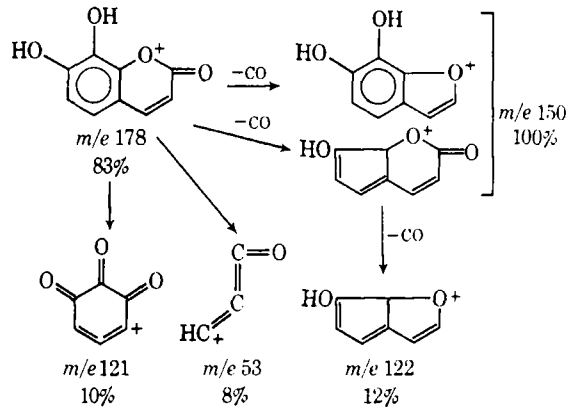
Compound A, giving color reactions for phenols, has the elementary composition of $C_9H_6O_4$ (mol. wt. 178.0282; theoretical 178.0266), and the UV spectral characteristics of a coumarin: λ_{max}^{EtOH} 224 (log ε 4.2), 262 (log ε 3.9), and 326 nm. (log ε 4.05) [literature (3) data for 7,8-dihydroxycoumarin: λ_{max}^{EtOH} 260 (log ε 3.8) and 327 nm. (log ε 4.1)].

Compound A was identified as 7,8-dihydroxycoumarin (daphnetin) from the mass spectrometry, IR, and NMR data.

Mass spectral findings can be rationalized as follows (Scheme I). The fragmentation pattern and relative mass peak abundance are very similar to those of umbelliferone (4), each corresponding *m/e* having an additional value of 16 (for the added oxygen).

The IR spectrum shows absorptions at 3360 (OH), 1665 (lactone), 1580, and 1490 (aromatic) cm.$^{-1}$.

A comparison of the chemical shifts and splitting pattern of the



Scheme I

three coumarins reported in the literature supports the structure as being dihydroxycoumarin (Table I).

The splitting of two aromatic protons into doublets (J = 9.5 Hz.) in the NMR spectrum shows them to be *ortho* to each other. Proton 4 in coumarins without substitution at C-5 resonates at δ7.65–7.77 [umbelliferone (5), herniarin (6), 7-geranyl-8-hydroxycoumarin (7), and scoparone (8)]. Substitution at C-5 with oxygen would have caused a downfield shift of proton 4 as in limettin (δ7.95) (9) and sibricin (δ7.95) (9); since the two aromatic protons of A (5 and 6) are coupled, the only positions left for the two hydroxyl groups are 7 and 8. Compound A is, therefore, 7,8-dihydroxycoumarin (daphnetin).

Several species of daphne have been found to contain daphnin, which is 7-daphnetin-β-glucoside (10, 11). Compound B, however, melts sharply at 229–230° compared to 215–217° (12) or 223–224° dec. (13, 14) for daphnin. Also, $[\alpha]_D^{24}$ is −92° compared to $[\alpha]_D^{22}$ −123.9° for daphnin (13). It must be concluded that in B the glucosidic linkage is at C-8 rather than at C-7. For verification, Compound B was peracetylated. The NMR spectrum of the acetate showed five acetyl groups and a downfield shift of proton C-6 by δ0.35, identical in magnitude to the shift experienced by the same proton in salicylic acid upon acetylation.

EXPERIMENTAL[2]

Isolation of A and B—Powdered dry root of *D. papyracea* was extracted with alcohol (95%), the extract was concentrated under reduced pressure, and the concentrate was poured in a thin stream into a large volume of water. The precipitated mass was separated by filtration and the filtrate was concentrated under reduced pressure. A 10% solution of neutral lead acetate was added to the concentrated liquid. A yellow precipitate (a) was filtered from the solution (b). Substance A was isolated from the yellow precipitate (a) by treatment of its aqueous alcoholic suspension with hydrogen sulfide gas, concentration of the resulting solution, and subsequent crystallization.

Filtrate (b) was treated with a 10% solution of basic lead acetate, yielding a pale-yellow precipitate (c) from which Compound B was isolated in the same way as was A from precipitate (a).

Hydrolysis of Compound B—Fifty milligrams of B was refluxed for 1 hr. with 20 ml. of 1% sulfuric acid, and the solution was then neutralized with barium carbonate and filtered.

TLC of the filtrate revealed a phenolic compound identical to that of Compound A. The adsorbent was silica gel G. The solvent systems were: (*a*) ethyl acetate–pyridine–water (3.6:1.0:1.15), (*b*) chloroform–methanol (60:40), and (*c*) butanol–acetic acid–water (4:1:1). The detectors were: (*a*) ferric chloride solution and (*b*) Pauly's reagent (15).

Chromatograms of the same filtrate gave spots of identical chromatographic behavior and color reactions as those of glucose. Two systems were used: (*a*) absorbent: silica gel G; solvent system: chloroform–methanol (60:40); and (*b*) absorbent: silica gel G buffered with sodium acetate; solvent system: acetone–water (90:10). The detector was aniline–diphenylamine–phosphoric acid (15). The nonaromatic portions of the NMR spectra of peracetylated Compound B and peracetylated glucose were virtually identical except for the presence of three protons at δ2.36 (aromatic acetyl group) in Compound B and three protons at δ2.10 (C-1 acetyl group) in peracetylated glucose.

Compound A was isolated from the filtrate. Admixture of this and A originally isolated from the plant gave no melting-point difference.

The presence of the β-glucosidic linkage in Compound B was shown by hydrolyzing B with emulsin at 24° for 2 hr. and identifying the released glucose.

Table I—NMR Spectral Data

Compound	Chemical Shift (δ) and Splitting Pattern H-3	H-4	H-5	H-6
Compound A ($CDCl_3$ + 30% dimethyl sulfoxide) (100 MHz.)	6.18 d	7.67 d	6.92 d	6.88 d
Umbelliferone ($CDCl_3$) (5)	6.26 d	7.77 d	7.42 m	6.89 m
Herniarin ($CDCl_3$) (6)	6.23 d	7.65 d	7.40 m	6.87 m
7-Geranyl-8-hydroxycoumarin ($CDCl_3$) (7)	6.25 d	7.65 d	7.38 m	6.88 m

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▲ To whom inquiries should be directed.

[2] IR spectra were determined in Nujol on a Perkin-Elmer 137, NMR spectra were determined in $CDCl_3$ on a Varian HA-100 spectrometer (Varian AG, Switzerland) and a Varian A 56/60 spectrometer (University of Ibadan, Nigeria), and mass spectra were determined on a Hitachi RMV 6 H mass spectrometer.