326. *A New Galactoside of Luteolin.*

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Chaerophyllum sylvestre has yielded a new glycoside, identified as a galactoside of luteolin.

During an investigation of possible alkaloidal constituents of hedge-row plants, the flowers and seed-pods of *Chaerophyllum sylvestre* (sheep's parsley) were studied. No basic crystalline material was found, but from the extracts there was isolated a yellow crystalline *compound*, m. p. 260° (decomp.), which appeared to be a glycoside and which was named chaerophyllin.

Extraction of the flowers and seed pods with dilute acetic acid, followed by basification, yielded an amorphous solid, whence continuous extraction with ethanol yielded a pale yellow crystalline substance (0·1—0·3% of the plant weight). Since the alkaloid content had been the original aim of the investigation, attention had been focussed on the plant towards the end of its flowering season. The material obtained in later stages of this investigation (from seed capsules only) proved to be unstable if kept, possibly owing to enzymes present in the plant at this time.

Chaerophyllin, after extraction, was insoluble in dilute acids and soluble in alkalis, yielding a deep yellow solution. It gave a greenish-brown ferric coloration. Its analysis was complicated by the presence of ethanol and water of crystallisation, but these were removed by prolonged heating in a vacuum at 160° and the empirical formula $C_{21}H_{20}O_{12}$ was obtained.

Hydrolysis with dilute hydrochloric or sulphuric acid afforded a yellow solid, m. p. 330° (decomp.), which, although insoluble in aqueous acids, dissolved in dilute sodium hydroxide yielding a deep yellow solution. Treatment of this solid with magnesium and alcoholic hydrogen chloride gave a deep pink coloration, discharged by alkali, a reaction indicative of a flavone, flavanol, or flavanone. An alcoholic solution gave a greenish ferric coloration suggesting a pyrocatechol structure. The filtrate from the hydrolysis was shown to contain galactose by comparison of the osazone with an authentic specimen.

The aglycone, which analysed as $C_{15}H_{10}O_6,H_2O$, formed a tetra-acetate, m. p. 226°. Hydrolytic fission with alcoholic potassium hydroxide gave an acidic and a ketonic fraction. The acidic fraction was obtained in amount too small to be identified, though it gave a green ferric coloration reminiscent of protocatechuic acid. The ketonic fraction gave a crimson 2 : 4-dinitrophenylhydrazone, which was identified as 3 : 4-*dihydroxyacetophenone* 2 : 4-*dinitrophenylhydrazone* by comparison with an authentic sample.

The m. p.s and analyses of the aglycone and its derivatives, its reactions, and its degradation product indicated that it was luteolin (5 : 7 : 3′ : 4′-tetrahydroxyflavone). The ultra-violet absorption spectrum, determined in ethanol, showed maxima at 2570 and 3530 A. (log ε_{molar} 4·21 and 4·30, respectively) and a minimum at 2860 A. (log ε_{molar} 3·93) in very good agreement with the values found for luteolin by Skarzynski (*Biochem. Z.*, 1939, **301**, 150) [max., 2580 (4·22) and 3550 (4·28); min., 2850 (3·86) A.].

An authentic specimen of luteolin was obtained through the courtesy of Professors F. Challenger and W. Bradley of Leeds, to whom are due our warmest thanks. Comparison, by the method of mixed m. p.s, of the aglycone with luteolin and of the corresponding tetra-acetates completely confirmed the identification. There was, however, insufficient material to attempt any determination of the position of attachment of the galactose moiety. The authors wish to draw attention to the incorrect values for the absorption spectrum of luteolin given by Shibata and Kimotsuki [*Acta Phytochim.* (*Japan*), 1922, **1**, 99].

Experimental.

Extraction.—The flowers and seed capsules of *Chaerophyllum sylvestre*, collected in June, were extracted (approximately 10 hours) with 4% acetic acid at 100°, and the solution filtered from the solid residue. After concentration in a vacuum to a small volume (100—150 ml.) and removal of deposited solid by centrifugation, the solution was basified with ammonium hydroxide (*d* 0·88), and the thick greenish-yellow precipitate was collected by centrifugation, washed several times with distilled water, pressed on a tile, and dried overnight (yield, 2—3%). The powdered, dry solid was then exhaustively extracted in a Soxhlet apparatus with ethanol. The residue on further extraction with acetone, chloro-

form, and light petroleum yielded no more material and was discarded. The deep-yellow ethanol solution was filtered and concentrated to a small volume. A yellowish and somewhat gummy precipitate separated from the cooled solution and was collected (yield from the extraction, 5—10%, *i.e.*, 0·1—0·3% of the plant material taken). Boiling the precipitate with 90% acetic acid gave a solution from which, on cooling, *chaerophyllin* separated as very pale yellow needles, m. p. 237° (decomp.). For analysis, it was recrystallised from aqueous ethanol (50%), whence it formed pale yellow, hair-like needles, m. p. 260° (decomp.) (Found : C, 50·9, 50·9, 51·3; H, 5·45, 6·2, 5·3. $C_{21}H_{20}O_{11},C_2H_5OH,2\frac{1}{2}H_2O$ requires C, 51·2; H, 5·75. Found, on a sample dried in a high vacuum at 150° : loss, 13·1; C, 54·1; H, 5·1. $C_{21}H_{20}O_{11},H_2O$ requires loss, 13·5; C, 54·1; H, 4·7. Found, on a sample dried further in a vacuum at 160°; loss 4·5; C, 56·1; H, 5·1. $C_{21}H_{20}O_{11}$ requires loss, 3·9; C, 56·3; H, 4·5%).

Chaerophyllin was soluble in dilute sodium hydroxide, yielding a yellow solution, and gave a greenish-brown ferric coloration. It was insoluble in dilute, but dissolved in concentrated, acids.

Hydrolysis of Chaerophyllin.—Chaerophyllin (100 mg.) was boiled under reflux for 1 hour with 10% sulphuric acid (3 ml.); the suspension became a deeper yellow colour. After the solution had cooled, the precipitate was collected and washed with water, the filtrate and washings being reserved for the identification of the sugar residue, the presence of which was indicated by positive Fehling's and Molisch's reactions. The precipitate, on crystallisation from aqueous ethanol, formed small yellow needles, m. p. 330°, undepressed on admixture with an authentic sample of luteolin (Found : C, 59·1, 59·2; H, 3·8, 3·75. Calc. for $C_{15}H_{10}O_6,H_2O$: C, 59·3, H, 3·95%. Ultra-violet light absorption in ethanol : maxima at 2570 and 3530 A., log $\varepsilon_{max.}$ 4·21 and 4·30, respectively). The product was soluble in concentrated mineral acids and in dilute alkalis, but insoluble in water, dilute acids, and sodium carbonate solution. With alcoholic ferric chloride it gave a green colour, indicative of a phenolic compound of pyrocatechol type, and with magnesium and alcoholic hydrogen chloride a bright pink colour typical of a flavone.

Acetylation.—The aglycone (100 mg.) was heated at 105° for 15 minutes with acetic anhydride (1 ml.) and anhydrous sodium acetate (300 mg.). After cooling, the mixture was poured into an excess of water, triturated, and set aside overnight. The precipitate was then collected, washed, dried, and recrystallised from ethanol, forming long, silky needles, sintering at 210—212° and melting at 226° (Found, C, 60·8; H, 4·0. Calc. for $C_{15}H_6O_6(CO{\cdot}CH_3)_4$: C, 60·6; H, 4·0%). The mixed m. p. with tetra-acetyl-luteolin (prepared similarly from authentic luteolin) was identical.

Hydrolytic Fission.—The aglycone (100 mg.) was heated under reflux for 3 hours with alcoholic potassium hydroxide (4 ml.; 50%). The solution was acidified with sulphuric acid and then treated with excess of sodium carbonate. The filtered solution was continuously extracted with ether, the extract dried and evaporated, and the very small residue of oil taken up in ethanol and treated with an ethanolic solution of 2 : 4-dinitrophenylhydrazine sulphate. The small amount of red precipitate was recrystallised from a very small quantity of ethanol, m. p. 246—247° (decomp.), undepressed on admixture with an authentic sample of 3 : 4-dihydroxyacetophenone 2 : 4-dinitrophenylhydrazone (see below).

The residue from the continuous ether extraction was acidified and again extracted continuously with ether. After drying and evaporation, the residue was a very small amount of an oily solid, which gave a greenish ferric coloration and dissolved in sodium hydrogen carbonate with effervescence. Attempted sublimation was unsuccessful, and no crystalline product was isolated.

Identification of Galactose.—The aqueous filtrate from the hydrolysis of the glycoside was treated with excess of barium hydroxide solution and then with carbon dioxide to remove the barium. The filtered solution was concentrated in a vacuum to a small volume (approx. 1 ml.) and treated with an aqueous mixture of phenylhydrazine and acetic acid. On warming the mixture to 100°, a yellow precipitate began to separate after 9 minutes. After being heated for ½ hour, the mixture was cooled, and the precipitate collected, washed, and recrystallised from ethanol, yielding sheaves of yellow, hair-like needles, characteristic of galactosazone, m. p. 198°, and undepressed on comparison with an authentic sample.

3 : 4-*Dihydroxyacetophenone* 2 : 4-*dinitrophenylhydrazone.*—This *derivative* was prepared in the usual way from 3 : 4-dihydroxyacetophenone (Rosenmund and Lohfert, *Ber.*, 1928, **61**, 2601) and crystallised from ethyl acetate as crimson prisms, m. p. 247° (decomp.) (Found : C, 50·4; H, 3·5. $C_{14}H_{12}O_6N_4$ requires C, 50·6; H, 3·6%).

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