Pratensein. 5,7,3′-Trihydroxy-4′-methoxyisoflavone

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The isolation of pratensein, a new isoflavone from red clover, is described. On the basis of microdegradative and paper chromatographic evidence it is shown to be 5,7,3′-trihydroxy-4′-methoxyisoflavone. Its synthesis using the ethoxalylation method also is described.

In the course of an investigation in this laboratory on the detection and estimation of the known estrogenic isoflavones in red clover (*Trifolium pratense*), paper chromatographic and ultraviolet spectral evidence suggested the presence of other isoflavones as minor constituents.[1] One of these has now been isolated in milligram quantities and found to be a new isoflavone. Its constitution is shown to be 5,7,3′-trihydroxy-4′-methoxyisoflavone (I), and the name pratensein is given to this substance. This paper describes the isolation, structural determination, and synthesis of pratensein. Part of this work already has been reported briefly.[2]

Pratensein occurs in clover in concentration of the order of a few milligrams per cent, in the presence of much larger quantities of other isoflavones, notably biochanin A (5,7-dihydroxy-4′-methoxyisoflavone) and formononetin (5-hydroxy-4′-methoxyisoflavone).[1,3] No selective method for its concentration from ethanol extracts of clover was available. After removal of the large proportion of fatty material and chlorophyll pigment, the bulk of the major isoflavones had to be removed by crystallization and countercurrent extraction. Pratensein was concentrated from the resulting material by absorption chromatography on a silica gel column. Fractions containing pratensein were then chromatographed as bands on sheets of thick paper in two successive solvent systems. By this means 40 mg. of pratensein was obtained from *ca.* 36 kg. of fresh clover worked up in two batches. Later it was found that a better yield was obtainable starting from dried plant material.

Pratensein gives a pale pink color on treatment with sodium amalgam in ethanol followed by concentrated hydrochloric acid. Analytical results agreed with a molecular formula $C_{16}H_{12}O_6$ or $C_{16}H_{14}O_6$, consistent with a flavonoid structure. The ultraviolet spectrum in ethanol (λ_{max} 263, inflections 285, 325; λ_{min} 237 mμ) suggests that it is an isoflavone[4,5] and not a flavone,[5,6] flavanone,[6,7] or isoflavanone.[8] The presence of free hydroxyl groups situated at the 5- and 7-positions is indicated by bathochromic shifts in ethanolic aluminum chloride and in ethanol saturated with sodium acetate.[9] These conclusions were confirmed by the infrared spectrum (KBr disk) which closely resemble spectra of other isoflavones with 5,7-dihydroxyl groupings (biochanin A and genistein, 5,7,4′-trihydroxyisoflavone). All show two O—H peaks at 3100–3500, chelated conjugated C=O frequencies near 1660, and intense aromatic bands in the region 1500–1630 cm.$^{-1}$. The presence of the methoxyl group[10] is indicated by bands near 2850 and 1028 cm.$^{-1}$ in common with the spectrum of biochanin A, but not that of genistein. Spectral and analytical evidence thus indicated that pratensein is a trihydroxymethoxyisoflavone.

The usual chemical method for the structural determination of isoflavones,[4,5] involving isolation and characterization of degradation products after alkaline hydrolysis under various conditions, was impracticable in the present study because of the very small amount of material available. The technique of microdegradation followed by identification of products by paper chromatography has been used by various workers[11-13] for the identification of known flavonoid compounds. Our experience with the isoflavones genistein and biochanin A indicated that these compounds can be degraded to phenols and phenylacetic acids under much milder conditions (dilute alkali, 100°) than those reported in the literature.[4,5] These observations were utilized in the chemical study of pratensein. Pratensein was degraded on a micro scale under mild conditions and the products worked up and examined by paper chromatography.

One of the products of alkaline hydrolysis of pratensein was identified chromatographically as phloroglucinol (II). This was interpreted as arising from ring A of pratensein and indicated that the 5- and 7-positions are the only ones hydroxylated in this ring. This left one hydroxyl and one methoxyl group to be assigned to the B ring.

A clue to the probable arrangements of these groups was given by the observation that pratensein gives a green color with ferric chloride, suggesting an *ortho* arrangement of these oxygen functions. This was confirmed on demethylation of pratensein with concentrated hydriodic acid. The main product was easily oxidized by Tollens reagent, gave a green color with ferric chloride, and chromatographic comparison with an authentic sample of orobol[14] (IV) showed them to be identical. These findings indicated that pratensein is orobol methyl ether, and there remained only to settle the question whether the methoxyl group is at the 3′- or 4′-position. The phenylacetic acids expected from alkaline degradation of these two structures,

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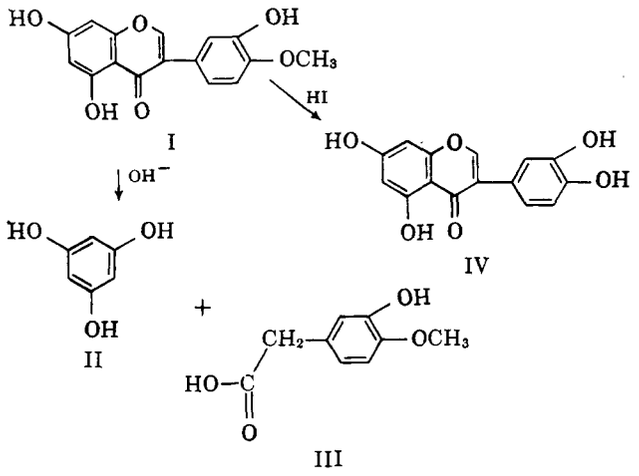
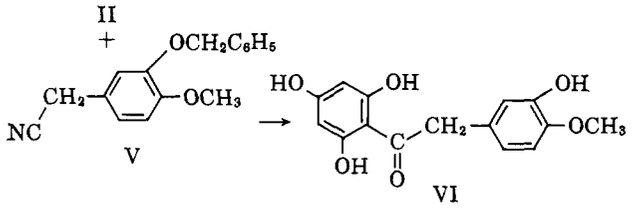
homovanillic acid and homoisovanillic acid, respectively, were, therefore, synthesized for comparison and homoisovanillic acid (III) was found chromatographically identical with one of the products from the alkaline degradation of pratensein.

On the basis of the previous evidence pratensein was assigned the constitution 5,7,3′-trihydroxy-4′-methoxyisoflavone (I).



This structure for pratensein has now been confirmed by synthesis. Of the various methods which are available for the synthesis of isoflavones, the method due to Baker and Ollis[15] involving the reaction of benzyl *o*-hydroxyphenyl ketones with ethoxalyl chloride is particularly suitable for the synthesis of isoflavones bearing several hydroxyl groups. The appropriate ketone in the present case (VI) was obtained by the



Hoesch reaction of phloroglucinol (II) and 3-benzyloxy-4-methoxyphenylacetonitrile (V). The nitrile (V), which had not been prepared previously, was obtained from O-benzylisovanillin *via* the following sequence of reactions[16]: aldehyde → substitutedrhodanine → thioketo acid → oximino acid → nitrile. The over-all yield of 3-benzyloxy-4-methoxyphenylacetonitrile from O-benzylisovanillin was *ca.* 30%.

The synthetic pratensein, obtained from the ketone (VI) by the ethoxalylation method, corresponded in melting point, infrared spectrum, and chromatographic behavior to the material isolated from red clover. The triacetyl derivatives of the natural and synthetic materials were identical.

Besides its presence in red clover, pratensein has been detected also in subterranean clover (*Trifolium subterraneum*), bringing the number of known isoflavones in these pasture species to five. The relative contribution of these constituents to the estrogenic activity of these forages has been discussed.[3]

In contrast to the common 4′-OH,3′-OCH_3 substitution pattern, the 3′-OH,4′-OCH_3 pattern has been noted in naturally occurring flavonoid compounds in only a few cases.[17] This is the first record in an isoflavone.

Experimental

All melting points were taken on a Kofler block.

Chromatographic solvents: BeAW, benzene–acetic acid–water (125:72:3 by vol.); BAW, *n*-butyl alcohol–acetic acid–water (6:1:2 by vol.); BAm, *n*-butyl alcohol–5% aqueous ammonia (85:15 by vol.).

Isolation of Pratensein. Extraction of Fresh Clover.—Freshly harvested red clover (*ca.* 20 kg.) was immersed in 95% ethanol (126 l.) and the mixture heated to nearly boiling and left soaking for 4 days. The dark green extract was drained off and concentrated in a cyclone evaporator down to a thick aqueous suspension (3.5 l.). The dark fatty solid material was separated by centrifugation and washed with boiling water (total 1 l.). After drying in a vacuum desiccator, this solid (400 g.) was extracted in a Soxhlet apparatus with petroleum ether (b.p. 60°) to remove the bulk of chlorophyll and lipid material. Further quantities of these substances were removed by washing the residue with successive portions of benzene (total 1 l.). The defatted material was extracted with boiling ethyl acetate (four 500-ml. portions) and the extract concentrated under reduced pressure to about 400 ml. The solid precipitated (15 g.), consisting mainly of formononetin and biochanin A, was filtered off and the mother liquor was evaporated to yield a dark green powder (15 g.).

Removal of Major Isoflavones by Partition.—The previous ethyl acetate-soluble material was partitioned in a countercurrent fashion in ten separating funnels each containing 450 ml. each of 70% ethanol and benzene. The fractions were analysed by two-dimensional paper chromatography in the solvent systems BeAW and 2 *N* ammonia.[1] Pratensein was found to be located in funnels 6, 7, and 8. The earlier fractions contained the bulk of the remaining lipids, green pigments, formononetin, and biochanin A.

Column Chromatography.—The fractions containing pratensein were combined and evaporated to dryness (yield, 1.77 g.). This was taken up in ethanol (15 ml.), the insoluble material (0.8 g.) was centrifuged off, and the solution absorbed onto 10 g. of silica gel (L. Light & Co., 100–200 mesh). The solvent was removed in a vacuum desiccator and the solid slurried with petroleum ether (b.p. 60°) and applied to the top of a column (3.5-cm. diameter) containing 200 g. of silica gel packed as slurry in petroleum ether. The column was developed with a linear gradient of 50–100% ether in petroleum ether. One hundred and fifty fractions of *ca.* 25 ml. each were collected. The fractions were evaporated and every fourth one examined by paper chromatography. Biochanin A was found in the earliest fractions (8–32). Pratensein, genistein, and other minor constituents appeared in fractions 40–64 with traces in the next twenty fractions.

Isolation of Pratensein by Paper Chromatography.—The column fractions containing pratensein were taken up in ethanol and applied as bands on twelve sheets of Whatman 3 MM paper, previously washed with 50% acetic acid followed by 95% ethanol for a total period of 48 hr. as for descending chromatography. The papers were chromatographed in BeAW and the pratensein bands (R_f 0.55), after location with ultraviolet light, were eluted with ethanol. The combined eluate (42 mg.) was then applied as bands to four sheets of washed paper and developed in the BAm solvent. Elution of the pratensein bands (R_f 0.33) with ethanol yielded 19 mg. of slightly colored solid, m.p. 260–263°. Repeated recrystallization from ethanol afforded colorless minute needles of pratensein, m.p. 272–273°.

Ultraviolet spectrum: (a) in ethanol, λ_{max} 263 (log ϵ 4.53), ~286 (log ϵ 4.16), λ_{min} 237 (log ϵ 4.17); (b) in ethanolic sodium hydroxide (0.01 *N*), λ_{max} 273, 330 (log ϵ 4.55, 4.16); (c) in ethanolic aluminum chloride (0.1%), λ_{max} 274 (log ϵ 4.53), ~310

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(log ϵ 3.94); (d) in ethanol saturated with sodium acetate, λ_{max} 273, 330 mμ (log ϵ 4.52, 3.96).

Infrared spectrum[18] (KBr): 3426, 3310, 3074, 2921, 2840, 1665, 1630, 1590, 1580, 1510, 1464, 1449, 1436, 1387, 1361, 1348, 1275, 1268, 1243, 1196, 1178, 1146, 1135, 1061, 1052, 1021, 997, 910, 880, 839, 826, 815, 808, 796, 784, 775, 760, 702, 650 cm.$^{-1}$.

Anal.[19] Calcd. for $C_{16}H_{12}O_6$: C, 64.0; H, 4.5. Found: C, 64.0; H, 4.0.

Pratensein gave a greenish color with 2% ferric chloride, which slowly changed to grayish purple. With sodium amalgam in ethanol, followed by addition of concentrated hydrochloric acid a pale orange-pink coloration was produced. On paper chromatograms it reacted slowly with silver nitrate[20] and gave a brownish orange color with diazotized sulfanilic acid[21]; R_f, BeAW 0.60, 2 *N* NH_3 0.52.

Isolation of Pratensein from Dried Clover.—Finely powdered oven-dried clover (3.8 kg.) was extracted with hot 95% ethanol (23 l.) and the soluble material processed as described before. After paper chromatography 70 mg. of pratensein was obtained as a light brown powder.

Structural Determination of Pratensein. Alkaline Degradation of Pratensein.—A solution of 4 mg. of pratensein in 1 ml. of 10% sodium hydroxide was heated under hydrogen on a water bath for 1.5 hr. After cooling, the reaction mixture was acidified with dilute hydrochloric acid and taken to dryness under reduced pressure. The residue was taken up in a small volume of absolute ethanol and the insoluble salt centrifuged off. The alcoholic extract was again evaporated to dryness, and the solid residue was extracted successively with ether and water. The two extracts were examined separately by paper chromatography. Similar products were present in both phases, but the concentration in ether was much higher.

Identification of Phloroglucinol.—This ether solution was chromatographed on Whatman no. 1 paper in the solvent systems BeAW, BAW, and 60% acetic acid. One of the reaction products separated was identified as phloroglucinol from R_f values (BeAW 0.07, BAW 0.75, 60% HOAc 0.68) and the following color reactions: ultraviolet light plus ammonia vapor, brilliant violet-blue fluorescence; diazotized sulfanilic acid, orange-yellow (acid), orange-brown (alkaline); vanillin–hydrochloric acid,[22] bright red; silver nitrate, slow reduction.

Demethylation of Pratensein.—About 5 mg. of pratensein was heated with 0.2 ml. of concentrated hydriodic acid (Sp. Gr. 1.72) on a boiling water bath for 3 hr. After dilution with an equal volume of water and addition of solid sodium metabisulfite, the reaction mixture was extracted (three times) with an equal volume of ethyl acetate. The combined organic phase was washed with a small proportion of water and taken to dryness. The residue was then taken up in 0.5 ml. of ethanol and analysed by paper chromatography (see following).

Identification of Orobol (IV).—Chromatography on Whatman no. 1 paper of this demethylation product in three solvent systems revealed two main components. One was identified as unchanged pratensein, and the other was identical with orobol in the following respects: R_f, BeAW 0.30, 60% HOAc 0.66, BAW 0.92; color reactions: ultraviolet, dark; diazotized sulfanilic acid, light orange (acid) and purple (alkaline); silver nitrate, reduced instantly; 2% ferric chloride, green-gray color.

Homovanillic Acid.—Homovanillic acid was synthesized from vanillin through the sequence of reactions: vanillin → O-benzylvanillin → 2-phenyl-4-(3′-methoxy-4′-benzyloxybenzal)-5-oxazolone → 4-benzyloxy-3-methoxyphenylpyruvic acid → 4-benzyloxy-3-methoxyphenylacetic acid → homovanillic acid. The benzylation of vanillin[23] and the transformations from O-benzylvanillin to 4-benzyloxy-3-methoxyphenylacetic acid[24] have already been described in the literature. The preparation of homovanillic acid from 4-benzyloxy-3-methoxyphenylacetic acid was carried out as follows: The benzyloxy acid (750 mg.) was heated for 1 hr. with a mixture of glacial acetic acid (15 ml.) and concentrated hydrochloric acid (5 ml.) in a water bath. The reaction mixture was then poured into two volumes of water and the whole taken to dryness under water pump vacuum. The solid residue was recrystallized from water (4 ml.) and washed with ether which removed most of the contaminating brown color. The resulting material was recrystallized from benzene, yielding colorless plates, m.p. 142° (lit.[25] m.p. 142, 139°).

Homoisovanillic Acid.—An attempt to synthesize this acid from isovanillin *via* the azlactone as was used for homovanillic acid was unsuccessful. Hydrolysis of the azlactone with sodium hydroxide[26] resulted in an intractable gum-like material. Homoisovanillic acid was obtained from homoveratric acid by partial demethylation wth concentrated hydrobromic acid as follows: A mixture of 1 g. of homoveratric acid and 1 ml. of hydrobromic acid (*ca.* 36%) was refluxed for 1.5 hr. The solution was evaporated under reduced pressure and the residue taken up in 5 ml. of water and treated with charcoal. The filtered solution, after concentration, deposited the acid on standing. Recrystallization from benzene yielded colorless crystals, m.p. 127–130° (lit.[27] m.p. 130–131°).

Identification of Homoisovanillic Acid in the Alkaline Degradation Product of Pratensein.—The synthetic homovanillic and homoisovanillic acids were chromatographed in BeAW, BAW, BAm, and 60% HOAc. The chromatographic properties (Whatman no. 1 paper) of these acids and those of one of the products from the previous alkaline degradation of pratensein are summarized in Table I.

TABLE I

	R_f				Diazotized sulfanilic acid	Silver nitrate
	BeAW	60% HOAc	BAW	BAm		
Homovanillic acid	0.65	0.65	0.75	0.14	Wine	++
Homoisovanillic acid	.68	.80	.90	.26	Reddish orange	+
Pratensein degradation product	.67	.82	.87	.22	Reddish orange	+

Acetylation of Pratensein.—A portion of the material isolated from dried clover (25 mg.) was heated with acetic anhydride and pyridine for 2 hr. at 100°. The product on crystallization from ethanol afforded broken prisms, m.p. 174–176°. This was later shown to be identical with synthetic pratensein triacetate.

Synthesis of Pratensein. O-Benzylisovanillin.—A mixture of isovanillin (30 g., 0.2 mole), benzyl chloride (37.8 g., 0.3 mole), finely powdered anhydrous potassium carbonate (15 g.) and potassium iodide (7.5 g.), and absolute methanol (100 ml.) was refluxed for 13 hr. After filtration from inorganic materials and concentration under reduced pressure, the product was steam distilled yielding a gum-like residue which solidified on standing at 0°. The crude product (42.5 g.) was recrystallized from ethanol (45 ml.) to give colorless needles (34 g.), m.p. 62° (lit.[26] m.p. 63°). This procedure in our hands gave better yields than described earlier.[26]

3-Benzyloxy-4-methoxybenzalrhodanine.—O-Benzylisovanillin (39.6 g.), rhodanine[28] (21.8 g.), anhydrous sodium acetate (50 g.), and acetic acid (200 ml.) were heated to reflux in an oil bath. After about 5 minutes' refluxing, the product quickly separated and the contents of the flask became almost solid. The bath was maintained at about 140° and the mixture heated for a further 30 min. After cooling, the product was dispersed in 300 ml. of water, filtered, and thoroughly washed with water. The yield of the crude product, which melted at 221–226°, was almost quantitative (58 g.). Recrystallization was best carried out from pyridine–benzene (1:3), yielding minute yellow needles, m.p. 226–227°. A sample was dried over phosphorus pentoxide *in vacuo* for 24 hr. for analysis.

(18) The writer is grateful to Dr. B. Cleverley, Dominion Laboratory, D.S.I.R., New Zealand, for infrared spectra measurements.

(19) Analyses were by Dr. A. D. Campbell, University of Otago, New Zealand.

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Anal. Calcd. for $C_{18}H_{15}O_3NS_2$: C, 60.49; H, 4.23; N, 3.92. Found: C, 60.41; H, 4.33; N, 4.00.

3-Benzyloxy-4-methoxyphenylthiopyruvic Acid.—The prior substituted benzalrhodanine (14 g.) was suspended in 70 ml. of 15% sodium hydroxide containing 3 g. of sodium sulfide and heated under nitrogen on a vigorously boiling water bath. The suspension dissolved after *ca.* 10 min. and the heating was then continued for a further 10 min. The mixture was cooled in an ice bath, then acidified quickly with 80 ml. of ice-cold 10% hydrochloric acid. The precipitate immediately coagulated into a sticky lump which was collected, washed well with water, and dried in a vacuum desiccator. The material thus obtained (12 g.), m.p. 110–120°, was very difficult to purify by recrystallization, and the crude product was used for the next stage of the synthesis. Repeated recrystallization of a small sample from benzene, followed by recrystallization from methanol, gave yellow flat prisms, m.p. 155–156°.

Several attempts were made to obtain a purer product by varying slightly the conditions. Prolonging the hydrolysis for 30–40 min.[16] resulted in the formation of an oil, and the acid was contaminated with a large amount of gum. A shorter period of hydrolysis (10–15 min.)[29] gave products containing substantial amounts of unchanged starting material.

3-Benzyloxy-4-methoxyphenylpyruvic Acid Oxime.—To a solution of 6 g. of sodium in 280 ml. of ethanol was added a solution of 18 g. of hydroxylamine hydrochloride in 15 ml. of water. The hydroxylamine filtrate was added to 28 g. of crude thioketo acid obtained as before, and the solution was refluxed for 1 hr. After filtration from a small amount of insoluble material, the solvent was removed and the orange-red residue extracted with an excess of 5% sodium hydroxide (200 ml.). The alkaline extract was separated from a very small amount of white solid, cooled in ice, and acidified with 10% hydrochloric acid (180 ml.). The colored precipitate was filtered off, taken up in 240 ml. of boiling ethyl acetate–benzene (1:5), and filtered. On cooling at 0° and standing, the oximino acid separated as a white fluffy solid (13.1 g.), m.p. 155–156°, after recrystallization from the same solvent.

Anal. Calcd. for $C_{17}H_{17}O_5N$: C, 64.91; H, 5.44; N, 4.44. Found: C, 64.28; H, 5.40; N, 4.88.

3-Benzyloxy-4-methoxyphenylacetonitrile (V).—The prior oximino acid (18 g.) was heated with 150 ml. of acetic anhydride on a water bath. Effervescence began on slight warming but gradually ceased after 30 min. The mixture was then heated under reflux for 15 min. when further vigorous effervescence took place. The reaction mixture, after cooling, was filtered from a small amount of insoluble material and shaken vigorously with an equal volume of water. After cooling and standing, the colored precipitate (13.5 g.) was filtered off, washed with water, and dissolved in 750 ml. of ether. The ether solution was repeatedly washed with 5% sodium hydroxide (total, 200 ml.) followed by several portions of water. Most of the coloration was removed by this process. Evaporation of the ether left a nearly pure product; the yield was 12.9 g. The product can be recrystallized very satisfactorily from isopropyl ether, yielding colorless stout needles, m.p. 80°.

Anal. Calcd. for $C_{16}H_{15}O_2N$: C, 75.86; H, 5.97; O, 12.64. Found: C, 76.21; H, 6.28; O, 12.80.

3-Hydroxy-4 methoxybenzyl 2,4,6-trihydroxyphenyl Ketone (VI).—A mixture of anhydrous phloroglucinol[30] (6 g.), 3 benzyloxy-4-methoxyphenylacetonitrile (12 g.), and powdered, freshly fused zinc chloride (8 g.) in anhydrous ether (700 ml.) was saturated with dried hydrogen chloride for 6 hr. at 0°. After keeping at 0° for 6 days, the ether solution was decanted from the red oily layer which had separated. The oily layer was rinsed twice with dry ether, then heated on a water bath with 100 ml. of 0.5 *N* hydrochloric acid for 2.5 hr. under an atmosphere of nitrogen. After standing and cooling, the product was collected. The yield of reddish brown crude solid was 5.7 g. This material was used for the next stage of the synthesis. Recrystallization from water (480 mg. in 100 ml., charcoal) gave slightly colored broken prisms (182 mg.), m.p. 105° (resolidifying) and 196–199°. The ultraviolet spectrum in ethanol showed a maximum at 288 mμ and a minimum at 248 mμ.

Acetylation with acetic anhydride–pyridine (100°, 2 hr.) yielded the tetraacetate which had m.p. 168–170° after recrystallization from ethanol.

Anal. Calcd. for $C_{14}H_7O_5(OCH_3)(COCH_3)_4$: C, 60.24; H, 4.84; OCH_3, 6.77; $COCH_3$, 37.56. Found: C, 60.56; H, 5.08; OCH_3, 6.27; $COCH_3$, 38.11.

That the product from the previous Hoesch reaction was the ketone (VI) and not its 3′-benzyl ether was further confirmed by hydrolysis of the ketone with 5% sodium hydroxide (6.5 hr., 100°). Examination of the products by paper chromatography revealed the presence of homoisovanillic acid but not of O-benzylhomoisovanillic acid. The hydrolysis of a benzyl ether during the Hoesch reaction has been noted by other workers.[31]

Pratensein-2-carboxylic Acid.—The prior ketone (3.1 g.) was dissolved in dry pyridine (30 ml.) and ethoxalyl chloride (6.1 ml.) added slowly with shaking at 0°. After keeping at 0° for 3 days, it was poured into water (60 ml.) and extracted with chloroform (three 40-ml. portions). This solution on evaporation gave a dark gum. This product, presumably the 2-carbethoxypratensein, was not purified but was directly hydrolyzed by refluxing for 2.5 hr. with a mixture of 40 ml. each of 5% sodium carbonate and ethanol. After removal of the ethanol under reduced pressure, the mixture was diluted with water and extracted with ethyl acetate (200 ml.). The ethyl acetate liquor was extracted repeatedly with saturated sodium bicarbonate solution (four 50-ml. portions). On acidification with hydrochloric acid, the aqueous extract liberated initially a dark oily scum which floated to the surface and was removed. The remaining mixture on standing and cooling deposited fine yellow crystals which were collected; the yield was 2.0 g. Recrystallization from dilute ethanol gave yellow needles, m.p. 275–277°, with effervescence.

Anal. Calcd. for $C_{16}H_9O_7(OCH_3)\cdot H_2O$: C, 56.35; H, 3.89; OCH_3, 8.56. Found: C, 56.65; H, 4.02; OCH_3, 8.44.

Pratensein (I).—The prior isoflavone-2-carboxylic acid (1.4 g.) was divided into seven portions (*ca.* 200 mg.). Each portion was placed in an ignition tube and heated in a salt bath maintained at 280–290° for approximately 3 min. when decomposition was complete. The dark product was removed from the ignition tubes with hot ethanol and the solution concentrated and left to stand overnight. The fawny brown deposit was collected and the dark mother liquor treated with charcoal and evaporated to give more light brown solid. This material was repeatedly washed with sodium bicarbonate solution. Combined yield of crude product was 410 mg. This material was best purified by sublimation at 210–220° (0.5 mm.) followed by recrystallization from ethanol giving colorless minute needles, m.p. and m.m.p. 272–273°. The ultraviolet and infrared spectra of this material were identical with those of natural pratensein.

Anal. Calcd. for $C_{15}H_8O_5(OCH_3)$: C, 64.00; H, 4.03; OCH_3, 10.33. Found: C, 63.40; H, 4.18; OCH_3, 10.46.

The triacetyl derivative was prepared with acetic anhydride–pyridine by the normal procedure. The product was recrystallized from ethanol as colorless, long thin prisms, m.p. 175–177°. Mixture melting point with the acetate derivative of the natural material was 174–175°.

Anal. Calcd. for $C_{15}H_6O_5(OCH_3)(COCH_3)_3$: C, 61.95; H, 4.25; OCH_3, 7.28; $COCH_3$, 30.28. Found: C, 62.14; H, 4.42; OCH_3, 7.59; $COCH_3$, 30.53.

(29) *Cf.* "Hydrolysis of 2,4-dimethoxybenzalrhodanine," O. H. Emerson and E. M. Bickoff, *J. Am. Chem. Soc.*, **80**, 381 (1958).

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