ether gave a crystalline solid (156 mg., m.p. 70–85°). Recrystallization from methanol five times yielded cholestane-7-one XXIV as needles (32 mg., 8.0%), m.p. 115–117° $[\alpha]_D^{27}$ −51°.

*Anal.* Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found: C, 83.65; H, 11.96.

There was no depression in melting point upon admixture with a sample of cholestane-7-one (m.p. 118°, $[\alpha]_D$ −47°) prepared by the method of Windaus,[43] and the infrared spectra of the respective samples were identical.

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# Flower Pigments. Flavonoids from *Argemone mexicana linn.* (*Papaveraceae*)

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This paper reports details of the isolation in pure form of isorhamnetin (4′,5,7-trihydroxy-3′-methoxyflavonol) and two of its glycosides from the flowers of *Argemone mexicana*. The new glycosides have been identified as isorhamnetin-3-glucoside and isorhamnetin-7-diglucoside.

*Argemone*, a genus of prickly herbs, includes about twelve species. *Argemone mexicana* (English prickly poppy, Mexican poppy; Hind. Bharband, Satiyanashi) is the only species[1] found in India. It is native to tropical America but has become naturalized in India and runs wild all over the country. The yellow juice which exudes when the plant is injured has long been used in India as a medicine[2] for dropsy, jaundice, and cutaneous affections. It was also considered as a diuretic.[2] The seed oil was used in lamps and medicinally[1] in ulcers and eruptions. The seeds and the seed oil had also been employed as a remedy[2] for dysentery and other intestinal affections. The mustard oil, adulterated with argemone oil, has been established to produce symptoms resembling those of epidemic dropsy.[1]

The plant has been reported[3] to contain berberine and protopine, and the fatty acid content of the seeds has been investigated.[4] In a recent note[5] the results of our preliminary investigation of the coloring matter of the bright yellow flowers were described.

A free aglycone, m.p. 304–306°, and two glycosides, m.p. 165–167° and 208–210°, have been isolated from the flower extract of *A. mexicana*. The aglycone was found to be a flavonol by the appearance of a pink coloration on reduction[6] with magnesium and hydrochloric acid and a bright yellow coloration with Wilson boric reagent.[7] The methanolic solution of the aglycone was not oxidized by pentammine cobaltrichloride,[8] indicating the absence of two or more adjacent phenolic hydroxyl groups. Micro-Zeisel determination showed the presence of one methoxyl group. Methylation of the aglycone with dimethyl sulfate yielded a compound that melted at 151–152° and showed no depression in melting point on mixing with an authentic sample of the pentamethyl ether of quercetin. The above observations prove that the aglycone is a monomethyl ether of quercetin. The possibility of the aglycone having a methoxyl group at C-5 is ruled out as it does not show fluorescence in acetic anhydride.[9] A comparison of the melting points of the aglycone and its acetate with those of known 7-, 5-, 3′-, and 4′-monomethyl quercetins suggested its identity with isorhamnetin. This was confirmed by a comparison of ultraviolet and infrared spectra, by chromatography and $R_f$ values and by a mixed melting point with authentic isorhamnetin.

The glycosides, m.p. 165–167° and 208–210°, give positive[6] tests with magnesium and hydrochloric acid and sodium amalgam followed by acidification, indicating thereby the flavanone or flavonol nature (with C-3 blocked)[10] of the glycosides. The appearance of a yellow color with Wilson boric acid reagent[7] eliminates the possibility of the glycosides belonging to flavanone class. On the basis of the above color reactions both the glycosides have been considered as flavonol glycosides. Both of them

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(8) E. Wada, *J. Am. Chem. Soc.*, **78**, 4725 (1956).

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on hydrolysis gave the same aglycone, m.p. 304–306°, which was characterized as isorhamnetin as described earlier. The sugar has been characterized as glucose by $R_f$ value, co-chromatography and by formation of an osazone, m.p. 204–205°. The possibility of uronic acids[11] being the carbohydrate moiety was also taken into account, and it was found that uronic acids are not present as the sugar part of either of the glycosides.

The position of the sugar residue in the glycosides has been determined by their methylation followed by hydrolysis. The partial methyl ethers obtained in both the cases have been characterized by melting points and mixed melting points with authentic samples and by the preparation of their acetates. The glycoside, m.p. 165–167° on complete methylation, gave an uncrystallizable oily mass which on hydrolysis gave a product, m.p. 193°, which gave no melting point depression when mixed with an authentic sample of 3′,4′,5,7-tetramethylquercitin.[12] This proves the attachment of the sugar at C-3 of the aglycone. The quantitative estimation of sugar by Somogyi's copper micro method[13] showed the presence of one mole of glucose per mole of aglycone. The glycoside, m.p. 165–167° is, therefore, characterized as isorhamnetin-3-glucoside.

The fully methylated product from the glycoside, m.p. 208–210° gave on hydrolysis a product, m.p. 284–285°; on acetylation it gave a product, m.p. 174° (*cf.* lit.[14] m.p. 174–176°). It was characterized as 7-hydroxy-3′,4′,3,5-tetramethyl quercetin by melting and mixed melting points with an authentic sample. The formation of the above partial methyl ether of quercetin shows the attachment of glucose at C-7. The estimation of sugar by Somogyi's copper micro method[13] showed the presence of two moles of glucose per mole of aglycone. The glycoside, m.p. 208–210° is therefore identified as isorhamnetin-7-diglucoside.

## EXPERIMENTAL[15]

*Extraction of flavonoids.* Dried flowers of *Argemone mexicana* (500 g.) were extracted successively with petroleum ether (b.p. 40–60°) and ethanol (95%). The alcoholic extract was distilled under diminished pressure, the residue was taken up with water, and extracted repeatedly with *n*-butyl alcohol. The combined *n*-butyl alcohol extracts, on leaving overnight, deposited a yellowish brown solid which was filtered and marked "A." The filtrate on recovery of the solvent left behind a powdery residue of reddish brown color. The residue was dissolved in hot water, and the aqueous solution on treatment with neutral and basic lead acetate yielded a yellow bulky precipitate (B) and an orange precipitate (C), respectively. The filtrate on deleading showed no tests for flavonoids.

*Purification of flavonoids.* A 0.5-g. sample from B was dissolved in dry acetone (50 cc.) and the solution was then passed through a 60-mm. diameter column packed to a depth of 160 mm. with magnesium trisilicate. A highly fluorescent band, observed under ultraviolet light, was removed during the course of washing of the column. This was found to be nonflavonoid in nature. The two flavonoid bands (ultraviolet light) were eluted from the column with ethyl acetate saturated with water. As there was certain overlapping of the bands, the separation could not be accomplished effectively. The two fractions from B obtained by column chromatography were purified finally by paper chromatography on Whatman No. 3 filter paper according to the method of Ice and Wender,[16] and their homogeneity was established by chromatographic examination on Whatman No. 1 filter paper, using butanol-acetic acid-water (60:10:20) and employing both ascending and descending techniques. The solid from C on similar treatment revealed only one band in ultraviolet light. This was eluted with ethyl acetate–water. The solid obtained on recovery of the solvent gave, on repeated crystallizations from methanol, yellowish brown plates, m.p. 165–167°. On chromatographic examination it was found to be a single entity. The two fractions after several crystallizations from methanol gave minute yellowish brown plates, m.p. 165–167° and yellow shining needles, m.p. 304–306°.

*Characterization of the aglycone, m.p. 304–306°. Acetylation.* The aglycone (150 mg.) was heated under reflux with acetic anhydride (4.5 cc.) and fused sodium acetate (300 mg.) for 2 hr., poured on crushed ice, and left overnight. The solid was collected, washed with water, and dried. On crystallization from ethanol (charcoal), it gave colorless needles (140 mg.) m.p. 202–204° (*cf.* lit.[17] m.p. 203–204°). It showed no depression in melting point on admixture with an authentic sample of isorhamnetin acetate.

*Anal.* Calcd. for $C_{24}H_{20}O_{11}$: C, 59.50; H, 4.16. Found: C, 59.62; H, 4.44.

*Deacetylation.* The above acetate (100 mg.) was refluxed with alcohol and hydrochloric acid (1:1) on a water bath for 2 hr. The yellow precipitate was filtered, washed with water, and dried. On crystallization from methanol it gave shining yellow needles (40 mg.), m.p. 304–306° (*cf.* lit.[18] m.p. 306°). The mixed melting point with an authentic sample of isorhamnetin was found to be undepressed. The identity of the aglycone as isorhamnetin was further confirmed by comparison of $R_f$ values[8,17] ultraviolet[17,19] and infrared spectra.[19]

*Anal.* Calcd. for $C_{16}H_{12}O_7$: C, 60.76; H, 3.82. Found: C, 60.55; H, 3.91.

*Methylation.* The aglycone (200 mg.) in dry acetone (100 cc.) was refluxed with methyl sulfate (1 cc.) and freshly ignited potassium carbonate (3 g.) for 30 hr. and worked up in the usual manner. The solid residue on crystallization from methanol and then with ethyl acetate gave colorless needles, m.p. and mixed m.p. 152° (*cf.* lit.[17] m.p. 151–152°) with an authentic specimen of pentamethylquercetin.

*Anal.* Calcd. for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41. Found: C, 64.42; H, 5.38.

*Isorhamnetin-3-glucoside. Glycoside acetate.* The crystalline glycoside (300 mg.), anhydrous pyridine (4 cc.), and acetic

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(15) All the melting points recorded in this paper were taken on a Kofler hot microscopical stage and are corrected. The infrared spectra were taken in Nujol, using a double beam Perkin-Elmer Spectrometer Model 137 (infracord). The microanalyses were done by Drs. Weilers and Strauss, Oxford. The ultraviolet spectra were measured in ethanol using a Beckman spectrophotometer Model DU.

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(17) K. V. Rao and P. K. Bose, *J. Indian Chem. Soc.*, **36**, 358 (1959).

(18) V. Deulofeu and N. Schopflocher, *Gazz. Chim. ital.*, **83**, 449 (1953).

(19) G. E. Inglett, *J. Org. Chem.*, **22**, 189 (1958).

anhydride (4 cc.) were heated at 85–95° for 3 hr. and poured on crushed ice. The precipitate was filtered, washed, and dried. On crystallization from dilute ethanol it gave colorless needles, m.p. 135–136°.

*Deacetylation.* The glycoside acetate (100 mg.) was dissolved in 0.1*N* methanolic sodium methoxide (25 cc.) and kept at 0° for 24 hr., neutralized with dilute hydrochloric acid, and the precipitated solid collected and dried. On several crystallizations from methanol it gave minute yellowish brown plates, m.p. 165–167°.

*Anal.* Calcd. for $C_{22}H_{22}O_{12}\cdot 2H_2O$: C, 51.36; H, 4.669. Found: C, 51.05; H, 4.74.

*Hydrolysis.* The anhydrous glycoside (0.5 g.) was hydrolyzed by refluxing with 125 cc. of 0.6*N* hydrochloric acid. The hydrolysis appeared to be completed within a few minutes, but the refluxing was continued for 2 hr. to ensure complete hydrolysis. After leaving overnight, the aglycone was filtered, washed, and dried. On repeated crystallizations from methanol it gave yellowish brown plates, m.p. 304–306° (*cf.* lit.[18] m.p. 306°). The aglycone showed no depression in melting point on admixture with an authentic sample of isorhamnetin. Its identity as isorhamnetin was further confirmed by co-chromatography and comparison of ultraviolet and infrared absorption spectra.

*Anal.* Calcd. for $C_{16}H_{12}O_7$: C, 60.76; H, 3.82. Found: C, 60.68; H, 3.80.

The aglycone gave an acetate, m.p. 202–204° and a pentamethyl ether of quercetin, m.p. 151–152° (*cf.* lit.[17] m.p. 151–152°).

The filtrate from which the aglycone was removed was concentrated to a syrup in vacuum. The concentration was continued until the syrup was neutral to litmus paper. Glucose was identified by chromatography using the two solvent systems, *n*-butyl alcohol–acetic acid–water (40:10:50) and *n*-butyl alcohol–water–ethanol (60:28.5:16.5). The osazone of the sugar was prepared from the concentrate by the usual method; it melted at 204–205° and showed no depression in melting point when mixed with the authentic specimen. Somogyi's[13] copper micro method gave the value (0.44 cc.) which corresponds to 1 mole of sugar per mole of glycone.

*Location of the sugar position of the glycoside.* The methyl ether of the glycoside was prepared by refluxing a mixture of the glycoside (300 mg.), acetone (100 cc.), methyl sulfate (1.5 cc.), and freshly ignited potassium carbonate (4 g.) on a water bath until a few drops of the mixture showed no coloration with ferric chloride. After filtration the acetone was removed by distillation and the brownish oily mass was directly hydrolyzed by refluxing with 7% sulfuric acid for 2 hr. On leaving overnight a yellow powdery mass separated. It was filtered, washed, and dried. Several crystallizations from ethanol gave straw-colored needles melting at 193° (*cf.* lit.[12] m.p. 192–194). A mixture of this ether with 3′,4′,-5,7-tetramethylquercetin showed no depression in melting point. It gave an acetate, m.p. 160–162° (*cf.* lit.[12] m.p. 160°).

*Isorhamnetin-7-diglucoside.* The yellow solid A which separated on leaving the *n*-butyl alcohol extract overnight gave yellow microscopic needles, m.p. 202–205°, from methanol; the m.p. rose to 208–210° after several crystallizations. The glycoside gave an acetate as colorless needles, m.p. 148–150°. The deacetylation of the glycoside acetate employing the procedure described earlier gave the original glycoside, m.p. 208–210° (methanol).

*Anal.* Calcd. for $C_{28}H_{32}O_{17}$: C, 52.5; H, 5.00. Found: C, 52.7; H, 4.98.

On hydrolysis the glycoside gave an aglycone, m.p. 304–306°, characterized as isorhamnetin as described earlier. The chromatographic examination of the filtrate showed the presence of glucose only. This was confirmed by the formation of an osazone, m.p. 204–206°.

*Estimation of sugars.* The anhydrous glycoside (46.1 mg.) was hydrolyzed by refluxing for 2 hr. with 2% sulfuric acid. After cooling overnight, the aglycone was filtered, dried, and weighed (20.6 mg.). The ratio of the aglycone to the glycoside is 44.7%, and this ratio indicates the presence of 2 moles of sugar per mole of aglycone.

Somogyi's[13] copper micro method gave the value (1.64 cc.) which also corresponds to 2 moles of sugar per mole of aglycone.

The position of the sugar was determined by methylation followed by hydrolysis, whereupon straw-colored needles, m.p. 284–285°, on several crystallizations from ethanol were obtained. This, on admixture with an authentic specimen of 3,3′4′,5-tetramethylquercetin, showed no depression in melting point. It gave an acetate, m.p. 174° (*cf.* lit.[14] m.p. 174°).

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