temperature, the specific conductance was measured. New weighed portions were added and dissolved, the specific conductance being measured after each addition.

Results and discussion. The experimental data are shown in Fig. 1 which gives the specific conductance as a function of the molality of the solutions of water and 1,1′-dianthrimide (Di). To facilitate comparison the concentrations of the latter reagent were divided by three.

From previous work on the sulphuric acid solvent systems it is known that many organic substances, *e.g.* anthraquinone, behave as bases,[4] that water is completely ionized,[5] and that the conductance of solutions of substances reacting as bases is mainly due to the HSO_4^- ions formed by the protolytic reaction.[6]

From Fig. 1 it is seen that one molecule of 1,1′-dianthrimide produced a conductance corresponding to three molecules of water. This result was explained by assuming the reaction

$$Di + 3H_2SO_4 = DiH_3^{3+} + 3HSO_4^-$$

For solutions of anthraquinone in sulphuric acid Hammett and Deyrup [4] showed that one molecule of both reagents participated in the protolytic reaction, the proton being attached to one of the carbonyl groups.

In the present system it was considered as highly probable that two of the three protons were attached to two of the four carbonyl groups, and that the third proton was attached to the imino group.

Previous authors [7] have noted that the colour of solutions of 1,1′-dianthrimide changed by varying the strength of concentrated sulphuric acid. In acids of low strength the solutions were but faintly coloured, while in strong acid they were green, the intensity of the colour increasing with the strength of acid.

This change may be explained by assuming that the first proton is taken up by the most basic group, the imino group, and that the resulting ion only produces a faint colour. By increasing the strength of the acid additional protons are taken up by the chromophore carbonyl groups, this resulting in strongly coloured solutions.

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3-Piperidino-Chromanone-(4)

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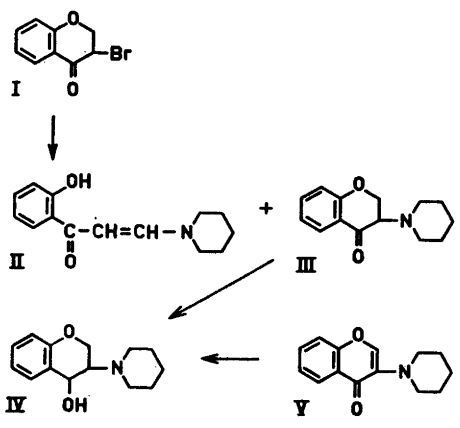
Earlier, Colonge and Guyot [1] described the reaction of 3-bromochromanone-(4) (I) with piperidine resulting in a yellow solid with m.p. 117°, and suggested it to be 3-piperidino-chromanone-(4) (III). This reaction has been reinvestigated. The isolated compound was not III, but was shown by its melting point (122 – 122.5°), ultraviolet and infrared spectra to be identical with *o*-hydroxy-*β*-piperidino-acrylophenone (II) obtained according to Winter and Hamilton [2] after treatment of chromone with piperidine. It can therefore



be assumed that the first step in the sequence of the above reaction consists in the formation of chromone with subsequent production of II. However, as the formation of III could not be excluded, the treatment of I was performed with the calculated amount (2 moles) of piperidine in dry benzene. After removal of the main product II, the expected piperidino-chromanone III could be isolated as its hydrochloride in poor yield. The reaction and isolation processes were followed by the ultraviolet absorption spectrum.

Catalytic hydrogenation of III resulted in 3-piperidino-chromanol-(4) (IV) which proved to be identical with material obtained by hydrogenation of 3-piperidino-chromone-(4) (V) on basis of the I.R. spectrum of the amino alcohol in KBr pellets. No attempt has been made to establish the stereochemistry of IV. The structure of III has been verified by its NMR-spectrum.

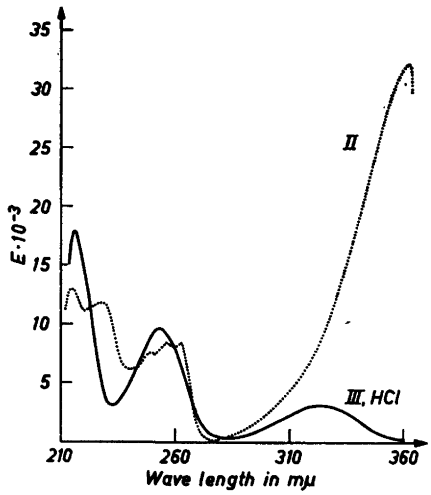


Fig. 1. Ultraviolet spectra of II and III, HCl in 96 % C_2H_5OH (Beckmann DK-2).

Experimental. Technical assistance: Sonja Jaccard. Analyses by G. Cornali and W. Egger of these laboratories. Melting points were taken in open glass capillaries in a Hershberg apparatus.

3-Piperidino-chromanone-(4) (III) and *o-hydroxy-β-piperidinoacrylophenone (II).* To a stirred solution of 73 g of 3-bromo-chromanone-(4)[3] (I) in 360 ml of dry benzene, 55 g of piperidine were added while the temperature was kept at about 20°. Piperidine hydrobromide began to separate within a few minutes. After stirring for several hours the reaction mixture was allowed to stand for 16 h at room temperature. Piperidine hydrobromide (50 g) was filtered off and washed with benzene. The combined filtrates were evaporated *in vacuo* on a water bath not exceeding 28°, the residue was treated with 1 l of petroleum ether, and the insoluble crude acrylophenone II (56 g; m.p. 117–119.5°) removed by filtration. Recrystallization from ethanol gave pure II; m.p. 122–122.5° (Lit.[2]: 123–124°); ultraviolet spectrum; *cf.* Fig. 1; the I.R. spectrum in KBr pellets was identical with that of authentic material.[2] (Found: C 72.80; H 7.46; N 6.02. Calc. for $C_{14}H_{17}NO_2$ (231.2) C 72.70; H 7.41; N 6.06). To the mother liquor was added 19 ml of 8.8 N hydrochloric acid in ethanol, and the mixture was evaporated *in vacuo*. To a solution of the residue in 110 ml of isopropanol 300 ml of ether was slowly added, precipitating 3-piperidino-chromanone-(4) hydrochloride (III, HCl) (7 g; m.p. 157° (decomp.)). Recrystallization from chloroform/ether raised the m.p. to 163–164° (decomp.) Ultraviolet spectrum, *cf.* Fig. 1; infrared band at 5.90 μ (carbonyl).* (Found: C 62.91; H 6.78; Cl 13.20; N 5.21. Calc. for $C_{14}H_{17}NO_2$ HCl (267.8) C 62.80; H 6.78; N 5.23; Cl 13.24).

3-Piperidino-chromanol-(4) (IV). (a) A solution of 2 g of 3-piperidino-chromone (V)[2] in 80 ml of 0.2 N hydrochloric acid and 0.4 g of 10 % Pd on carbon powder catalyst was hydrogenated at room temperature. After 2 h nearly the calculated amount of hydrogen (2 moles) had been consumed. The catalyst was removed by filtration, and the crude chromanol V (1.7 g) precipitated by addition of 40 ml of saturated sodium bicarbonate solution. Recrystallization from isopropanol, and drying *in vacuo* yielded 1.7 g of the carbinol; m.p. 124.5–125.5°. (Found: C 72.19; H 8.24; N 5.99. Calc. for $C_{14}H_{19}NO_2$ (233.3) C 72.07; H 8.21; N 6.00).

Hydrochloride. M.p. 201.5 (ethanol/ether). (Found: 62.14; H 7.52; Cl 13.08; N 5.19. Calc. for $C_{14}H_{19}NO_2$ HCl (269.77) C 62.33; H 7.47; Cl 13.14; N 5.19).

(b) A solution of 1 g of 3-piperidino-chromanone-(4) hydrochloride (III, HCl) in

* A very weak band was present at 6.03 μ, possibly attributable to an impurity which could not be removed by further recrystallizations.

40 ml of 0.1 N hydrochloric acid and 0.2 g of 10 % Pd on carbon powder catalyst was hydrogenated, and V isolated as described under (a). Hydrogen absorption: 1 mole. Yield: 0.62 g; m.p. 124.5–125.5°, unchanged after recrystallization from isopropanol. The IR spectrum in KBr pellets was identical with that of IV prepared as described under (a). (Found: C 72.19; H 8.35; N 5.97).

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Molecular Weight of Renin Determined by Sephadex Gel-filtration

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The molecular weight of the kidney enzyme renin is unknown. It has not been possible to determine the molecular weight because renin has still not been prepared in a purity allowing "classical" molecular weight determination. With the introduction of Sephadex, molecular weight determination by gel-filtration seems possible, even when the substance (*i.e.* renin) for which the molecular weight is sought, is applied to the column in a crude preparation.[1]

In order to correlate the molecular weight of renin with other substances with known molecular weight, three "tracer" substances were employed: (1) ^{131}I-labelled human γ-globulin with a molecular weight of 160 000,[2] (2) ^{125}I- or ^{131}I-labelled human albumin with a molecular weight of 69 000,[3] and (3) recrystallized pepsin with a molecular weight of 35 000.[4]

Two preparations of renin were applied to gel filtration: (1) Goldblatt-renin, step V (Biochemical Nutritional Company), and (2) a hog renin preparation made by acetone-extraction of freeze-dried, ether fractionated kidney powder followed by kaolin adsorption and ammonium sulphate fractionation. Renin was estimated according to the method of Skeggs, Kahn, and Marsh.[5]

Two types of Sephadex were employed: G-200 and G-100, and two different column sizes, *viz.* one with a diameter of 21 mm and a length of 800 mm and another with a diameter of 13 mm and a length of 1050 mm. Elution was performed with a 0.5 M phosphate buffer, pH 6.0. Most of the runs were performed at 4°C but some at 20°C.

Until now 17 runs have been done. In all these experiments we have found the same pattern of elution of renin (and "tracer" elements), independent of renin preparation (Goldblatt-renin or our own preparation), type of gel, column size, number of "tracers" in one run *etc.* Renin is eluted between albumin and pepsin.

On the assumption that this method is applicable to renin preparations the molecular weight of renin is — determined by interpolation — between 42 000 and 49 000.

We are now performing experiments with other tracer substances in order to make a more detailed study of the molecular weight of renin.

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