9. *Synthetical Experiments on the Nature of Betanin and Related Nitrogenous Anthocyanins. Part II.*

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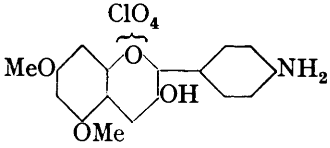
THE properties of the aminoflavylium salts described in Part I were such as to lend support to the view that they may be closely related to the anthocyanin-like pigments of the *Chenopodiaceae*, but, in general, the red colour of the solutions of the hydrochlorides was changed to yellow or orange on the addition of hydrochloric acid in moderate excess and this is not a property of betanin (cf. Willstätter and Schudel; Schudel, Diss., Zürich, 1918). We have now found that 4′-aminoflavylium salts, containing two or three hydroxyl groups in the positions (3, 5, 7) corresponding to those hydroxylated in the anthocyanidins, possess a colour, stability to cold mineral acids, and behaviour with alkalis quite similar to the respective properties of the pigment of the beet. The latter is, however, the more unstable towards hot mineral acids and, on the other hand, it appears to be more resistant to the action of nitrous acid than is any of the synthetic salts.

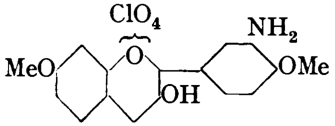
These divergencies indicate that, although betanidin is undoubtedly a member of the aminoflavylium salt group, we are not yet in possession of the true synthetic model for it; possibly *N*-methylation will enable the gap to be bridged.

In extending the series of aminoflavylium salts we first applied the methods described in Part I to the preparation of methoxylated derivatives, more highly substituted than heretofore.

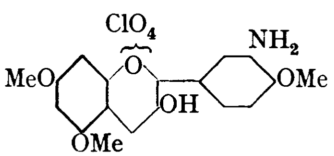
The properties of (I) showed that sufficient auxochromes had been introduced into appropriate positions to give the necessary blueness of tone of red solutions in acidified alcohol. The effect of a change in the position of the amino-group (from 4′ to 3′) was then studied and it was found that the salts (II) and (III) did not resemble betanin very closely in respect of the colour of acid solutions. (Methylation of hydroxyl groups does not alter the visible colour of acid alcoholic solutions of the anthocyanins in large degree.)



(I.)



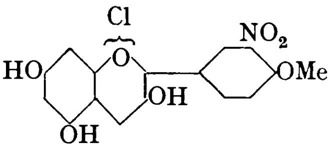
(II.)

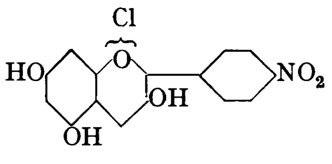


(III.)

Comparison of (III) with a related salt (IV) containing a nitro-group and of both with similar 4′-substituted salts and with pelargonidin and cyanidin derivatives showed that the amino- and nitro-groups exert hypsochromic and bathochromic effects, respectively, when occurring in position 3′, but these influences are much more efficient when either of the groups occupies position 4′ as in (V) and (VIII).

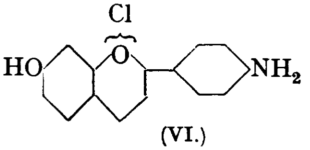
(IV.)



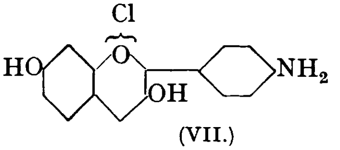


(V.)

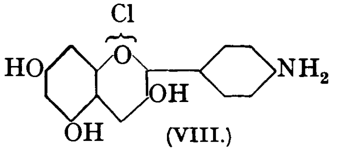
Although considerable experimental difficulties were encountered, it was ultimately found possible to prepare a series of hydroxylated salts (VI, VII, VIII) and of these the pelargonidin analogue (VIII) exhibits the closest similarity to the beet-pigment.



(VI.)



(VII.)



(VIII.)

EXPERIMENTAL.

3-*Nitro-4-methoxybenzoyl Chloride*.—A mixture of 3-nitroanisic acid (1 mol.) and PCl_5 (1 mol.) was heated on a steam-bath for 6 hr. and then freed from $POCl_3$; the residue crystallised from light petroleum (sparingly sol.) in long white needles, m. p. 52° (Found: C, 44·4; H, 2·6; N, 6·9; Cl, 16·1. $C_8H_6O_4NCl$ requires C, 44·5; H, 2·8; N, 6·5; Cl, 16·5%).

ω-*Diazo-3-nitro-4-methoxyacetophenone*.—A solution of 3-nitro-4-methoxybenzoyl chloride (20 g.) in $CHCl_3$ (50 c.c.) was added during 15 min. to ethereal CH_2N_2 (from 20 c.c. of nitrosomethylurethane); a vigorous evolution of N accompanied the separation of pale yellow crystals of the diazo-ketone. After 12 hr. the cryst. material was collected, washed with Et_2O (yield, 20 g.; m. p. 138—139°), and recrystallised from EtOH, forming rectangular plates, m. p. 139° (Found: C, 49·0; H, 3·5; N, 18·0. $C_9H_7O_4N_3$ requires C, 49·0; H, 3·2; N, 19·0%). The diazo-ketone is easily sol. in org. solvents with the exception of light petroleum and it exhibits the characteristic behaviour of diazo-compounds towards I_2, acids, and alc. NaOH; exposure to direct sunlight causes it to become bright yellow.

3-*Nitro-ω-acetoxy-4-methoxyacetophenone*.—The diazo-ketone (15 g.) was mixed with sufficient AcOH to make a thick paste and warmed on a water-bath for 20—30 min. until the evolution of N appeared to be complete; the liquid was then boiled for 2 min. The cooled solution was poured slowly with stirring into cold H_2O; the *ω-acetoxy-ketone*, which separated (15 g., m. p. 110°), crystallised from EtOH in large yellow rectangular plates, m. p. 110°, readily sol. in EtOH, C_6H_6, or EtOAc and sparingly sol. in light petroleum. It reduced Fehling's solution readily (Found: N, 5·8. $C_{11}H_{11}O_6N$ requires N, 5·5%).

3-*Amino-ω-acetoxy-4-methoxyacetophenone*.—The nitro-ketone (2 g.), suspended in boiling H_2O (50 c.c.), was added to a boiling solution of $FeSO_4,7H_2O$ (50 g. in 200 c.c. H_2O) and aq. NH_3 (about 10 c.c., *d* 0·880) was slowly introduced with efficient stirring. Finally, the alkaline solution was boiled 2—3 min. and filtered hot. The *amino-ketone* separated quickly from the filtrate in yellow plates, m. p. 110°, and 119—120° after recrystn. from hot H_2O (easily sol.) (Found: C, 59·3; H, 5·8; N, 6·7. $C_{11}H_{13}O_4N$ requires C, 59·2; H, 5·8; N, 6·3%). The substance is readily sol. in the simple alcohols, and reduces Fehling's solution slowly in the cold, rapidly on warming. It is easily sol. in HCl aq. and can be diazotised and then couples with β-naphthol to an azo-compound.

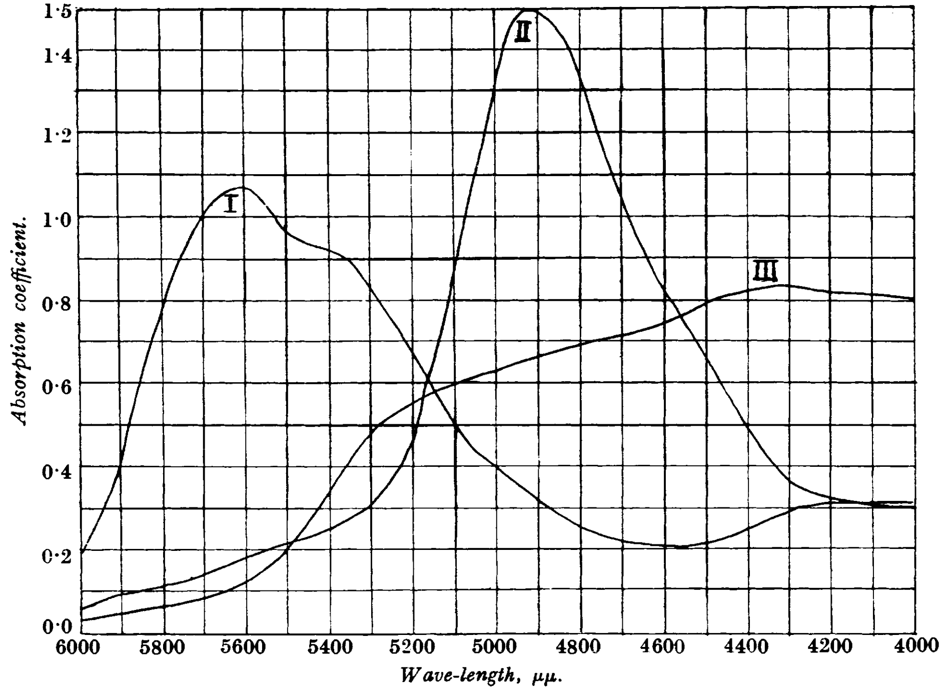
4′-*Amino-3-hydroxy-5 : 7-dimethoxyflavylium Perchlorate* (I).—A solution of 2-hydroxy-4 : 6-dimethoxybenzaldehyde (0·92 g.) and 4-amino-ω-acetoxyacetophenone (0·97 g.) in conc. HCl (150 c.c.) was saturated with HCl at 0° during 6 hr., and left over-night. The chloride was sparingly sol. and 0·7 g. was collected; 20% $HClO_4$ aq. (150 c.c.) was added to the filtrate and, after 24 hr., the solution was filtered and the cryst. perchlorate washed with Et_2O and dried. Recryst. from AcOH, or from 20% $HClO_4$ aq., the salt formed microscopic needles (Found in material dried in vac.: C, 49·3; H, 4·0; N, 3·5; Cl, 9·0. $C_{17}H_{16}O_8NCl,H_2O$ requires C, 49·1; H, 4·3; N, 3·4; Cl, 8·6%). The salt gives a permanganate-coloured solution in EtOH and, more readily, a cherry-red solution in MeOH. The aq. solution is red, and the addition of conc. HCl to aq. or alc. solutions produces an orange colour. Addition of Na_2CO_3 aq. and NaOH aq. to the alc. solutions gives greenish-yellow colorations and NaOAc aq. precipitates the purplish-red colour-base.

3′-*Amino-3-hydroxy-7 : 4′-dimethoxyflavylium Perchlorate* (II).—A Schiff's base, prepared by condensing equiv. quantities of 2-hydroxy-4-methoxybenzaldehyde and 3-amino-ω-acetoxy-4-methoxyacetophenone in abs. EtOH, was dissolved in conc. HCl, and the solution saturated with HCl, but no evidence of pyrylium salt formation was forthcoming. The direct condensation, however, succeeded. 3-Amino-ω-acetoxy-4-methoxyacetophenone (1·12 g.) and 2-hydroxy-4-methoxybenzaldehyde (0·78 g.) were dissolved in just sufficient conc. HCl, and HCl passed into the solution. The pyrylium salt was formed immediately and the solution was saturated at 10° during 6 hr. and then kept for 12 hr. The chloride was rather sparingly sol. in the conc. HCl, so more acid was added and the solution warmed to dissolve as much as possible of the solid. It was then filtered and 20% $HClO_4$ aq. added to precipitate the cryst. *perchlorate*; after 24 hr., this was collected and recrystallised from much AcOH, forming bright red needles (Found in material dried in a high vac.: C, 47·5; H, 4·6; N, 2·7; Cl, 9·9. $C_{17}H_{16}O_8NCl,2H_2O$ requires C, 47·1; H, 4·6; N, 3·3; Cl, 8·2%). The salt is sol. in MeOH and EtOH to bright red solutions, which become orange on addition of conc. HCl. It is very sparingly sol. in H_2O and cold dil. HCl, but it is sol. in the hot acid to give an orange solution. On addition of Na_2CO_3 aq. and NaOH aq. the coloured alc. solution becomes yellow and pptn.

of a brownish pseudo-base occurs. The colour is restored on addition of acid. With NaOAc aq. the purplish-red colour-base is pptd.

3'-Amino-3-hydroxy-5 : 7 : 4'-trimethoxyflavylium Perchlorate (III).—A solution of 3-amino-ω-acetoxy-4-methoxyacetophenone (1·12 g.) and 2-hydroxy-4 : 6-dimethoxybenzaldehyde (0·96 g.) in conc. HCl (150 c.c.) was saturated with HCl at 10° during 6 hr., then kept over-night and filtered. Sufficient 20% $HClO_4$ aq. was added to ppt. the *pyrylium* salt as bright red needles, which were recrystallised from 20% $HClO_4$ aq. (400 c.c.) (Found in air-dried material: C, 46·6; H, 4·7; N, 3·1; Cl, 8·4. $C_{18}H_{18}O_9NCl,2H_2O$ requires C, 46·6; H, 4·8; N, 3·0; Cl, 7·7%). The salt is easily sol. in MeOH to a red solution, less readily in EtOH to give a bluish-red solution. Addition of conc. HCl to the alc. solutions changes the colour to orange. The salt is almost insol. in cold dil. aq. acids. Addition of Na_2CO_3 aq. and NaOH aq. to the alc. solutions gives yellow solutions and some pptn. of pseudo-base; with NaOAc aq. the colour-base is pptd.

FIG. 1.



The curves I, II, and III were obtained with the substances so indicated in the text above; I was examined in 0·5*N*/10⁴-, and II and III in *N*/10⁴-solution in 0·1% HCl–MeOH in a cell of 20 mm.

3'-*Nitro*-3 : 7-*dihydroxy*-5-*benzoyloxy*-4'-*methoxyflavylium Chloride*.—*O*-Benzoylphloroglucinaldehyde and 3'-nitro-ω-acetoxy-4-methoxyacetophenone did not condense in dry Et_2O solution by means of HCl; the aldehyde separated and no sign of pyrylium salt formation could be detected. The salt was formed by means of 80% $H{\cdot}CO_2H$ aq. and could be obtained on the addition of dry Et_2O to the solution. Still better results followed the use of dry EtOAc as the solvent. *O*-Benzoylphloroglucinaldehyde (1·3 g.) was dissolved in the minimum vol. of EtOAc and the ω-acetoxy-ketone (1·2 g.), when added, dissolved; the solution was cooled to 0° and saturated with dry HCl and then kept over-night. The chloride (1·7 g.) was obtained in bronze rectangular plates. The crude salt (0·4 g.) was dissolved in EtOH (50 c.c.), and conc. HCl (21 c.c.) added slowly to the hot solution; on cooling, the *pyrylium* salt crystallised in crimson clusters of plates (0·25 g.) (Found in air-dried material: C, 54·3; H, 4·0; N, 2·6; Cl, 6·7. $C_{23}H_{16}O_8NCl,2H_2O$ requires C, 54·6; H, 4·0; N, 2·8; Cl, 7·0%). The salt is sparingly sol. in dil. HCl and glac. AcOH; it is sol. in MeOH and EtOH to orange-red solutions. Addition of conc. HCl to the alc. solution gives an orange coloration. On the addition of NaOAc aq. a yellow solution is obtained; with Na_2CO_3 aq. a greenish-brown solution and pptn. of the colour-base result, and with NaOH aq. a greenish-brown solution is obtained; the addition of mineral acid to these solutions restores the orange-red colour of the pyrylium salt.

*3′-Nitro-*3 : 5 : 7-*trihydroxy-*4′-*methoxyflavylium Chloride* (IV).—The benzoyl derivative (above) was hydrolysed by means of EtOH–HCl. The benzoyl salt (4 g.) was dissolved in EtOH (250 c.c.), conc. HCl (63 c.c.) added, and the solution heated on the steam-bath for 12 hr.; the EtOH was then distilled, and the residual solution kept until crystn. was complete. The crude material was dissolved in EtOH, and the filtered solution saturated with HCl; the *chloride* separated as dark purplish-red crystals (Found in air-dried material: C, 52·1; H, 3·8; N, 3·3. $C_{16}H_{12}O_7NCl$ requires C, 52·5; H, 3·3; N, 3·8%). The salt is freely sol. in MeOH and EtOH to red solutions, which are considerably more intensely coloured than those of the benzoyl derivative. These solutions on the addition of conc. HCl become orange. Addition of Na_2CO_3 or NaOH to the alc. solutions gives dark purplish-green colorations and addition of NaOAc gives a purplish-red solution, from which, on keeping, the colour-base separates.

*4′-Nitro-*3 : 5 : 7-*trihydroxyflavylium Chloride* (V).—A solution of 4 : 6-dihydroxy-2-benzoyloxybenzaldehyde (2·59 g.) and 4-nitro-ω-acetoxyacetophenone (2·1 g.) in dry EtOAc was cooled in ice-water, and saturated with dry HCl during 6 hr. The chloride separated in purplish-brown crystals, which were collected (2·6 g.) after 12 hr. This salt is characterised by very sparing solubility in MeOH and EtOH, acetone, AcOH and BuOH. It is also insol. in hot H_2O and in dil. or conc. mineral acids. On the addition of Na_2CO_3, NaOH or NaOAc to the alc. solution, a weak pink coloration is obtained.

The insolubility of this salt renders the removal of the benzoyloxy-group by hydrolysis difficult, but it was ultimately effected by means of alc. HCl. Nitrodihydroxybenzoyloxyflavylium chloride (2 g.) was suspended in a solution of conc. HCl (25 c.c.) in abs. EtOH (100 c.c.), and the whole refluxed for 18 hr.: the solid was then shown by analysis to consist partly of unchanged material and partly of the debenzoylated salt.

From the filtrate the debenzoylated *salt* slowly separated in plates, which were washed with dry Et_2O (Found in air-dried material: C, 51·0; H, 4·1; Cl, 10·8. $C_{15}H_{10}O_6NCl,H_2O$ requires C, 50·9, H, 3·4; Cl, 10·1%). The salt is almost insol. in hot H_2O and dil. and conc. HCl; in MeOH and EtOH it dissolves to brown solutions, which become pink on the addition of Na_2CO_3, NaOH or NaOAc.

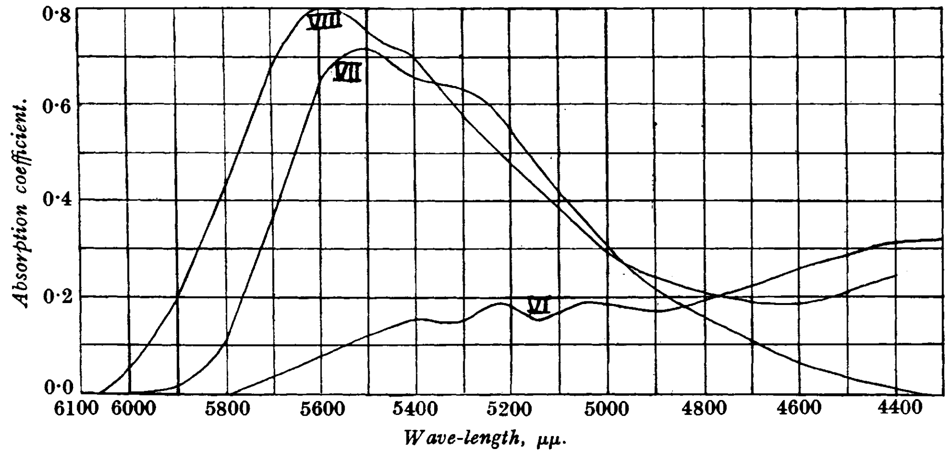
*4′-Amino-*7-*hydroxyflavylium Chloride* (VI).—4-Aminoacetophenone (0·68 g.) was dissolved in conc. HCl, β-resorcylaldehyde (0·69 g.) added, and HCl passed into the cooled stirred solution for 6 hr. The yellowish ppt. was then collected, washed with Et_2O, and dried, becoming brick-red in colour; it was recrystallised from MeOH (yield, 0·5 g.) (Found in material dried in a high vac. over P_2O_5: C, 65·3; H, 4·9; N, 4·7. $C_{15}H_{12}O_2NCl$ requires C, 65·8; H, 4·4; N, 5·1%). The *salt* is sparingly sol. in H_2O to a pale red solution, and easily sol. in MeOH and EtOH to cherry-red solutions. On the addition of Na_2CO_3 aq. these solutions become brownish-yellow, but formation of pseudo-base is not observed; NaOH aq. also gives a brownish solution. On the addition of NaOAc aq. the red colour persists and no separation of colour-base occurs. On the addition of conc. HCl to aq.-alc. solutions a yellow coloration is obtained.

*4′-Amino-*3 : 7-*dihydroxyflavylium Chloride* (VII).—When a cooled solution of β-resorcylaldehyde (1·38 g.) and 4-amino-ω-acetoxyacetophenone (1·93 g.) in conc. HCl (30 c.c.) was saturated with HCl, pptn. of the Schiff's base occurred and the mixture became extremely viscous. Further conc. HCl (30 c.c.) was therefore added, and the mixture saturated during 3 days. The crude material was crystallised from MeOH and more was obtained on addition of Et_2O to the filtrate (Found in material dried at 110° in high vac. over P_2O_5: C, 62·4; H, 4·1; N, 4·7; Cl, 12·8. $C_{15}H_{12}O_3NCl$ requires C, 62·2; H, 4·2; N, 4·8; Cl, 12·3%). The dark greenish-black *salt* is easily sol. in H_2O and the simple alcohols to give blood-red solutions: addition of Na_2CO_3 to the alc. solution gives a greenish-brown solution but no pseudo-base; on the addition of NaOAc a brown colour-base, insol. in $CHCl_3$, is formed. Addition of conc. HCl to the alc. solution gives a brownish solution, which becomes yellow only at great dilution. The amino-group is easily diazotised.

*4′-Amino-*3 : 7-*dihydroxy-*5-*benzoyloxyflavylium Chloride*.—A solution of 4-amino-ω-acetoxyacetophenone (0·4 g.) and 4 : 6-dihydroxy-2-benzoyloxybenzaldehyde (0·52 g.) in conc. HCl (20 c.c.) was saturated with HCl; some of the benzoylphloroglucinaldehyde then separated, but gradually redissolved as the reaction proceeded. The solution was saturated for 12 hr. As addition of Et_2O did not ppt. the pyrylium salt, the liquid was evaporated to dryness in vac. over KOH, the residue dissolved in MeOH, and the purple pyrylium salt pptd. by addition of dry Et_2O. The material obtained by this process is a mixture of the benzoyl and the debenzoylated salt, and when the latter is required the crude material can be treated directly with KOH aq. in the usual manner, this being the best way to prepare the salt in quantity. When, however, the pure benzoyl derivative is required the condensation should be carried out in EtOAc. A solution of 4 : 6-dihydroxy-2-benzoyloxybenzaldehyde (1·3 g.) and 4-amino-ω-acetoxyacetophenone (0·97 g.) in dry EtOAc (200 c.c.) was cooled in ice and saturated with dry HCl during 6 hr. Immediately the gas was passed, some of the Schiff's base separated as the hydrochloride, which was insol. in EtOAc and was not converted into the pyrylium salt on standing. It is very stable and is not readily hydrolysed even on heating with dil. mineral acids.

After 48 hr. the solution was filtered and gave the *hydrochloride* of the Schiff's base (1·1 g.) (Found in air-dried material: C, 61·3; H, 4·5; N, 3·1; Cl, 7·7. $C_{24}H_{20}O_7NCl$ requires C, 61·3; H, 4·3; N, 3·0; Cl, 7·6%), and from the filtrate on addition of Et_2O the *pyrylium* salt (0·85 g.) was obtained (Found in air-dried material: C, 59·9; H, 4·6; N, 3·5; Cl, 9·1. $C_{22}H_{16}O_5NCl,2H_2O$ requires C, 59·3; H, 4·5; N, 3·1; Cl, 8·0%). This salt is difficultly sol. in H_2O, easily sol. in dil. HCl to give a reddish-violet solution, and very easily sol. in the simple alcohols to reddish-purple solutions. Addition of Na_2CO_3 aq. gives a green solution and NaOAc ppts. a green colour-base which is sol. in $CHCl_3$ and C_6H_6. On addition of conc. HCl to the alc. solutions the red colour persists and is changed towards orange only on the addition of a very large excess of the acid. The salt is readily diazotised.

FIG. 2.



4′-Amino-3 : 5 : 7-trihydroxyflavylium Chloride (VIII).—4′-Amino-3 : 7-dihydroxy-5-benzoyloxyflavylium chloride (0·5 g.) was added to cooled 8% aq.-alc. NaOH (5 c.c. H_2O and 7·5 c.c. EtOH), and the solution kept for 3 hr. at room temp., air being excluded by N and the mixture occasionally shaken. After addition of conc. HCl (10 c.c.), the whole was heated at 60° for 30 min., filtered, cooled, and kept in the ice-chest over-night. A dark purple *salt* separated in clusters of needles (0·35 g.), and this was washed with dry Et_2O (Found in air-dried material: C, 55·4; H, 3·9; N, 4·1; Cl, 11·3. $C_{15}H_{12}O_4NCl,H_2O$ requires C, 55·3; H, 4·3; N, 4·3; Cl, 10·9%). This salt is even more easily sol. than the benzoyl derivative in H_2O, dil. HCl, and the simple alcohols to give red-purple solutions; on addition of conc. HCl the red colour persists even at great dilution. Na_2CO_3 added to the alc. solution gives a green coloration, fading to greyish-mauve; with NaOAc a purple colour-base is obtained. The colour of the salt in acid solution is changed to brownish-yellow on the addition of $NaNO_2$.

The curves VI, VII, and VIII refer to the substances so denoted in the text; VI was examined in $N/10^4$-, VII in $0{\cdot}25N/10^4$-, and VIII in $0{\cdot}5N/10^4$-solution in 0·1% HCl–MeOH in a 20 mm. cell.

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