**Condensation of *o*-Nitrophenylacetimino Ether with *p*-Nitrobenzenesulfonyl Chloride.**—The normal method of condensation of pyridine failed to give clean products. *o*-Nitrophenylacetimino ether base (8.4 g.) was melted with *p*-nitrobenzenesulfonyl chloride (4.4 g.). Reaction was immediate and the temperature rose to 80°. Some ethyl chloride was evolved, as normally occurs.[8] The melt, after a few minutes at 90°, was extracted successively with ether and water and the residue (4.9 g.) m. p. 115° (incomplete) was recrystallized from alcohol. The mixture was taken up in boiling ethyl acetate (30 cc.) and the product which separated on cooling (0.5 g., m. p. 157–158°) was removed. After a crystallization from ethyl acetate this had m. p. 159° and was identified as *o*-nitrophenylacetamide (Calcd.: N, 15.55. Found: N, 15.4). The ethyl acetate liquors were concentrated and successive crops removed. These were crystallized from 1:1-benzene/light petroleum mixture to give the required ***p*-nitrobenzenesulfonyl-2-nitrophenylacetimino ether**, m. p. 123°; yield 2.55 g. *Anal.* Calcd. for $C_{16}H_{13}O_7N_3S$: N, 10.7. Found: N, 10.8.

**Attempted Reduction of Above.**—Two grams dissolved in acetone (50 cc.) was reduced catalytically in the usual way (0.1 g. of platinum oxide) giving an uptake of 12 H in sixty-five minutes. After filtration from catalyst, the acetone was removed at low temperature *in vacuo*. The residue had a strong indole-like odor and was soluble in ether. No pure product was isolated and it was clear that the sulfonyl group had been split off.

The author's thanks are due to the Directors of Messrs. May & Baker Ltd., England, for permission to publish this work, and to Mr. S. Bance for the semi-micro analyses.

## Summary

1. When 2-aminoindole is condensed with acetylsulfanilyl chloride, the sulfonyl residue enters in the 1 position.

2. A method of obtaining the isomeric 2-sulfanilyl derivative by use of appropriate sulfanilyl amidines or imino ethers is outlined.

3. It is suggested that this method might find other similar applications in this field.

DAGENHAM, ENGLAND RECEIVED AUGUST 24, 1944

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA]

# The Composition of the Solid Secretion Produced by Primula Denticulata[1]

BY WALTER C. BLASDALE

A distinguishing feature of a large percentage of the species of *Primula* is the secretion of white or yellow solids commonly designated by botanists as farina. They are produced by minute, two-celled, gland-tipped hairs found on the leaves, scapes, calyces, or more rarely the petals. The chemical nature of the secretion of *P. pulverulenta* was studied by Hugo Müller[2] who found it to consist of flavone associated with small amounts of a wax-like component. Later Brunswick,[3] by the use of a microchemical test devised by Klein,[4] found that flavone was present in the secretions of 25 of the species of *Primula* as well as three of the closely related genus *Dionysia*. This test involved the subjection of fragments of farina-bearing tissue or of minute portions of farina scraped from it, to the fumes of concentrated hydrochloric acid, at a temperature of 40°, in a sublimation ring. If flavone was present a microscopic examination of the tissue or the cover glass revealed bundles of fine white or yellow needle-like crystals.

Appreciable differences in the color and consistency of the secretions of the different species suggest that additional components, both colorless and colored, should be looked for. Macroscopic study of them is rendered difficult because of difficulty of procuring enough plants upon which they are found. The seed of many species is unobtainable, many are difficult to grow, and the yield of farina-bearing tissue per plant is very small. During the past fifteen years I have been able to grow sufficient plants of twenty-one of the farina-bearing species to make a partial study of their secretions possible. This paper is concerned chiefly with the secretion of *P. denticulata*. I desire to express my obligation to Mr. Charles Koch of this Laboratory for microcombustions of several of the products separated.

(1) Original manuscript received January 11, 1943.

(2) Müller, *J. Chem. Soc.*, **107**, 872 (1915).

(3) Brunswick, *Ber. Akad. Wiss. Wien.*, [1] **131**, 221 (1922).

(4) Klein, *ibid.*, [1] **131**, 23 (1922).

### Experimental Part

**Separation of Farina.**—Owing to the sticky nature of the secretion and the small amount of farina-bearing tissue available, mechanical separation of it was not feasible. The procedure finally adopted was to treat the dried and coarsely powdered leaves and flower heads for twenty minutes, at room temperature, with a three-fold volume of benzene and allow the filtered extract to evaporate spontaneously. The dry residue consisted of white crystals and green amorphous matter, the total equaling about 4% of the tissue used. The same procedure applied to non-farinate leaves of the same species yielded nearly 2% of a green sticky mass from which no crystals could be obtained even by use of a variety of solvents. It seems probable that none of the crystals in the extract from the farina-bearing tissue came from the tissue itself.

**Some Properties of Flavone.**—It was soon apparent that most of the crystalline material present in the benzene extract was flavone. In separating it from the other components constant use was made of a boiling solution of 6 *N* hydrochloric acid. Although acid addition products of several flavone derivatives have been isolated,[5] these compounds hydrolyze at once unless the concentration of the acids used in their preparation is left high. It was found that constant boiling hydrochloric acid dissolved 2.65 g. of flavone per liter but about 90% separated, on standing at room temperature, as long fine needles. This may be attributed to its low melting point as compared with those of its derivatives. By treating the benzene extract with large volumes of 6 *N* acid twice, the flavone was almost completely separated from the large amounts of plant pig-

(5) Perkin, *J. Chem. Soc.*, [2] **69**, 1439 (1896).

ments, waxes, etc. The resulting solution, as well as the crystals which separate on cooling, was lemon-yellow. Repeated recrystallization of the yellow crystals from the pure solvent finally gave white crystals of the same form. Small amounts of yellow coloring matter were recovered from the solutions from which the first crystals separated. The dry, white crystals, even at room temperature, slowly lost weight and became opaque, owing to the liberation of both water and hydrochloric acid. The opaque residue melted at 95°. The melted product, purified by crystallization from either hot petroleum ether or 60% alcohol, formed shorter white crystals which melted at 97°, the currently accepted melting point of flavone. Further, the fine white crystals from the acid solution, as well as the crystals which melted at 97°, responded to the tri-iodide test,[6] that is, gave a deep purple color when treated with a drop of a dilute solution of potassium tri-iodide. When the same process of separation and purification was applied to farina-bearing tissue derived from *P. malacoides* and *P. Mooreana* products were obtained which melted at 97°, gave molecular weights of 209, 232 and 240, and the following analyses.

| | *P. malacoides* (a) | *P. malacoides* (b) | *P. Mooreana* | Calcd. for $C_{15}H_{10}O_2$ |
|---|---|---|---|---|
| Carbon, % | 80.91 | 81.45 | 80.76 | 81.05 |
| Hydrogen, % | 4.36 | 4.06 | 4.92 | 4.54 |

Further, when the scapes and calyces of *P. pulverulenta* (the species studied by Müller) were subjected to the same procedure both the fine crystals from the acid solution and the purified product could not be distinguished from the corresponding products obtained from *P. denticulata*.

**The Composition of Flavone Hydrochloride.**—The fine white crystals, which were separated from hydrochloric acid solutions of the species studied, after drying, were found to decompose slowly, even at room temperature, unless kept in closed containers. Complete elimination of the solvent from which they separated without some decomposition was difficult to effect. Samples derived from three species were allowed to dry at room temperature and weighed at twenty-minute intervals until the rate of loss showed a decided drop. Three portions of each of these samples were weighed out for determination of (a) loss at 105°, (b) percentage of hydrochloric acid, found by adding water and titrating with 0.01 *N* sodium hydroxide, and (c) melting point in a sealed capillary. The results are tabulated.

| | *P. denticulata* | *P. malacoides* | *P. japonica* | $C_{15}H_{10}O_2 \cdot HCl \cdot H_2O$ |
|---|---|---|---|---|
| Loss at 105° | 18.88 | 19.85 | 17.44 | 19.70 |
| Percentage HCl | 13.60 | 12.81 | 13.03 | 13.19 |
| M. p., °C. | 132–134 | 134–135 | 134–135 | |

These figures indicate that the fine white crystals correspond to the formula $C_{15}H_{10}O_2 \cdot HCl \cdot H_2O$. It is noteworthy that when heated in a small, sealed capillary they behave like a one component system. Heated in an open capillary they began to melt as low as 107°. This is a compound to which Klein's test is due. Its long fine needles of uniform diameter are readily distinguished from the coarser, splinter-like crystals of flavone.

**Separation of a Dihydroxyflavone.**—By treating the dry amorphous mass, left after removal of flavone from the benzene extract, with a large amount of hot carbon tetrachloride and filtering while hot, a green solution was obtained. Evaporation gave some amorphous matter and a few crystals. The latter were purified by washing with petroleum ether and recrystallizing from either benzene or ethyl ether. The purified product, which I will designate as Pr. 228, consisted of orange-yellow, 6- or 8-sided crystals of short-columnar or tabular habit. Between crossed nicols the dominant face gave extinction parallel to the longer edge and marked birefringence. The dominant and adjacent narrow faces gave brilliant reflections. I am indebted to Mr. Adolph Pabst of the Department of Geology for measurements of the corresponding interfacial angles on two millimeter-long crystals. These results, in addition to the parallel extinction, indicate that the crystals are monoclinic, with a $\beta$ angle of 82°18′.

Heated in a sealed capillary Pr. 228 melted at 228°. Determination of its molecular weight, by lowering of the melting point of camphor, gave 269, 234, 265 and 246. Analyses of five mg. samples gave the following results.

| Sample | C, % | H, % |
|---|---|---|
| A, by the writer | 70.42 | 4.02 |
| B, by the writer | 70.68 | 3.81 |
| C, by Charles Koch | 70.98 | 4.12 |
| Calcd. for $C_{16}H_{10}O_4$ | 70.86 | 3.93 |

When treated with 0.5 *N* sodium hydroxide Pr. 228 slowly forms a deep yellow solution from which it is reprecipitated unchanged by neutralization. Acetylation of a 13-mg. sample, by means of acetic anhydride and sodium acetate, gave a white, crystalline compound which, after recrystallization from ethyl acetate, melted at 183–184°. By hydrolysis and distillation this product yielded 1.71 moles of acetic acid per mole. By dissolving Pr. 228 in glacial acetic acid, adding a small amount of strong sulfuric acid and allowing to stand for several days a red crystalline product separated. It yielded 24.90% of sulfuric acid; the compound $C_{15}H_{10}O_4 \cdot H_2SO_4$ should yield 27.84%.

The preceding analytical data, associated with the results obtained by treating Pr. 228 with sodium hydroxide and with acetic anhydride suggest that it is a dihydroxyflavone. Its orange-yellow color, the formation of a red addition product with sulfuric acid, which is a characteristic of compounds containing a $\gamma$ pyrone nucleus, and its insolubility in hot 6 *N* hydrochloric acid, confirm this suggestion. It differs from all of the eight dihydroxyflavones listed by Beilstein in its physical properties. Since it constitutes only about 0.15% of the selected farina-bearing tissue (about one-ninth that of flavone) further work leading to the determination of the position of the hydroxyl groups must await the accumulation of larger amounts of such tissue. Its occurrence in the farina rather than in the main leaf tissue was proven by allowing a few drops of chloroform to flow over an undried, farina-bearing leaf and allowing the solution to evaporate. The residue, examined under a microscope, showed, in addition to flavone, the easily identifiable crystals of Pr. 228. Neither flavone nor Pr. 228 could be separated from the benzene extract of dry non-farina-bearing leaves of this species.

**The Wax-like Components.**—The carbon tetrachloride extract left after removal of Pr. 228 formed a sticky green mass composed of plant pigments and the decomposition products resulting from treatment of them with hydrochloric acid. Small amounts of wax-like preparations, melting from 40 to 65°, were separated by means of dilute alcohol but they could not be resolved into pure compounds.

**Composition of the Farina of Other Species.**—The process described above was used, with several modifications, in studying the farina of the twenty other species. Flavone was separated in pure form from all of them and the available data indicate that it constitutes at least 75% of the secretions of all these species. Small amounts of two additional compounds were isolated. From *P. verticillata* a yellow crystalline substance, which melted at 153° and gave a molecular weight and composition corresponding to $C_{15}H_{10}O_3$, was obtained. From *P. florindae* a second yellow compound, which began to decompose at 185° and at 205° gave a black amorphous solid and a few white crystals was obtained. Pr. 228 was found in the secretion of *P. denticulata* only. Nine of the twenty-one species, namely, *P. auricula, capitata, denticulata, frondosa, japonica, malacoides, marginata, pulverulenta* and *verticillata* are included in the list found to give qualitative tests for flavone by Brunswick.[3] The remaining twelve, namely, *P. Beesiana, Bulleyana, burmanica, chungensis, Florindae, helodoxa, Jaffreyana, microdonta, Mooreana, pulchelloides, Sherriffae* and *sikkimensis* do not appear to

(6) Barger and Starling, *J. Chem. Soc.*, **107**, [I] 411 (1915).

have been studied chemically heretofore. Wax-like products were obtained in variable but small quantities from all of the 21 species. In none of these was the percentage found in excess of ten and some of it may have been derived from the epidermal cells rather than the secreting hairs.


## Summary

Flavone was found to form at least 75% of the farina of twenty-one species of primula, by heating with 6 *N* hydrochloric acid, filtering the hot solution, cooling to 20° and separating the $C_{15}H_{10}O_2 \cdot HCl \cdot H_2O$ formed. The farina of *P. denticulata* contained about 10% of an orange-yellow compound, $C_{15}H_{10}O_4$, which melted at 228° and behaved like an undescribed dihydroxyflavone. A second yellow compound, $C_{15}H_{10}O_3$, which melted at 153° was separated from *P. verticillata*. Varying amounts of wax-like components were found in all the twenty-one species.


BERKELEY, CALIF. RECEIVED OCTOBER 25, 1944

# NOTES

## Bis-(trimethylenediamino)-cupric Sulfate

BY LAWRENCE H. AMUNDSEN[1] AND LENA A. MALENTACCHI

This substance was prepared for use as a germicide in the treatment of surface tissues by iontophoresis after the desired combination of properties had not been found in a number of other copper compounds, including the corresponding one from ethylenediamine. A report on these tests is expected to appear soon.[2]

Bis-(trimethylenediamino)-cupric sulfate is obtained readily from trimethylenediamine and cupric sulfate, either anhydrous (blue) or as a monohydrate (pinkish-purple). The hydrate is the stable form under ordinary conditions but loses water slowly at 56° and promptly at 100°. At room temperature it dehydrates in a desiccator over calcium sulfate (Drierite). The hydrate absorbs no more water under the atmospheric conditions prevailing in the laboratory except in the most humid summer weather, when some samples gained as much as 4–5% in weight. It is very soluble in water.

### Experimental

**Trimethylenediamine.**—A solution was prepared from 882.4 g. (7.81 moles) of trimethylene chloride, 12636 cc. (187.4 moles) of ammonium hydroxide, and 7 liters of 95% ethanol and left standing in a stoppered bottle at room temperature (19–22°), samples being withdrawn at weekly intervals for determinations of ionizable chlorine (Table I). After five weeks the mixture was distilled to dryness under the vacuum of a water-jet pump (13 mm.).

TABLE I

YIELD OF CHLORIDE ION

| Reaction period, days | % Theor. |
|---|---|
| 7 | 33 |
| 14 | 63 |
| 21 | 82 |
| 28 | 88 |
| 35 | 92 |

(1) Present address: Department of Chemistry, University of Connecticut, Storrs, Conn.

(2) By Commander Armand J. Pereyra, Medical Corps, U. S. Navy.

After the addition of 1637 cc. (31.24 moles) of 50% sodium hydroxide the mixture again was distilled to dryness under vacuum. The distillate was collected in two fractions of 873 cc. and 720 cc., respectively. Water (220 cc.) was added to the residue and a third fraction was obtained by distilling to dryness again. The three fractions were saturated with sodium hydroxide. The upper layer was separated from each and dried over sodium hydroxide. The layers were separated again and more sodium hydroxide was added to the upper layers. This process was repeated until fresh sodium hydroxide remained unchanged when added to the products. Upon rectification through a 180-cm. column packed with glass helices, 161 g. of trimethylenediamine was obtained from the first fraction and 21.5 g. from the second, making a yield of 32% of the theoretical. No attempt was made to rectify the product from the third fraction because of its high viscosity and because of the low yield from even the second fraction. It presumably consisted largely of amines of higher molecular weight. The trimethylenediamine was collected at 48–50° at 20 mm. It boiled at 133° cor. at 754.5 mm.[3,4] The pressure was reduced to 5 mm. and the distillation was continued as long as any liquid would come over. When 21 cc. of material so obtained was rectified again in a semi-micro apparatus,[5] 9.4 g. of a product boiling at 128–131.5° at 20 mm. was obtained (b. p. 230.5° cor. at 760.2 mm.[3]). This compound is presumed to be bis-(γ-aminopropyl)-amine, which von Braun[4] reported that he obtained as a fraction boiling from 210–230°.

**Bis-(trimethylenediamino)-cupric Sulfate.**—A mixture of 15.4 g. (0.208 mole) of trimethylenediamine, 24.97 g. (0.1 mole) of C. P. cupric sulfate pentahydrate, and 15 cc. of water was boiled gently under a reflux condenser until all of the copper sulfate went into solution. The mixture then was placed in a vacuum desiccator over calcium chloride. From time to time the crust on the surface was broken up, and the drying was continued until there was no further loss in weight. The product was the monohydrate (Table II).

(3) The determination was made by the micro method described in Shriner and Fuson's, "Identification of Organic Compounds," second edition, John Wiley and Sons, Inc., New York, N. Y., 1940, p. 93.

(4) Fisher and Koch (*Ber.*, **17**, 1799 (1884)) reported 135–136° at 738 mm.; Putokhin (*Trans. Inst. Pure Chem. Reagents* (Moscow) No. 6, 10 (1927)), 135–136°; von Braun, *et al.* (*Ber.*, **70B**, 979 (1937)), 136–138°; Aspinall (THIS JOURNAL, **63**, 2843 (1941)), 131° at 760 mm.; Whitmore, *et al.* (*ibid.*, **66**, 725 (1944)), 138° at 735 mm. The higher boiling points may have been determined on samples that were not completely dry. Tests made during the present study showed that the boiling point was raised by the addition of a little water and, furthermore, that the apparent boiling point would be several degrees higher than reported above if the sample was exposed to the atmosphere of the laboratory for a few minutes during the determination.

(5) Weston, *Ind. Eng. Chem., Anal. Ed.*, **5**, 179 (1933).