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THE CONSTITUTION AND SYNTHESIS OF DAMASCENINE, THE ALKALOID OF NIGELLA DAMASCENA

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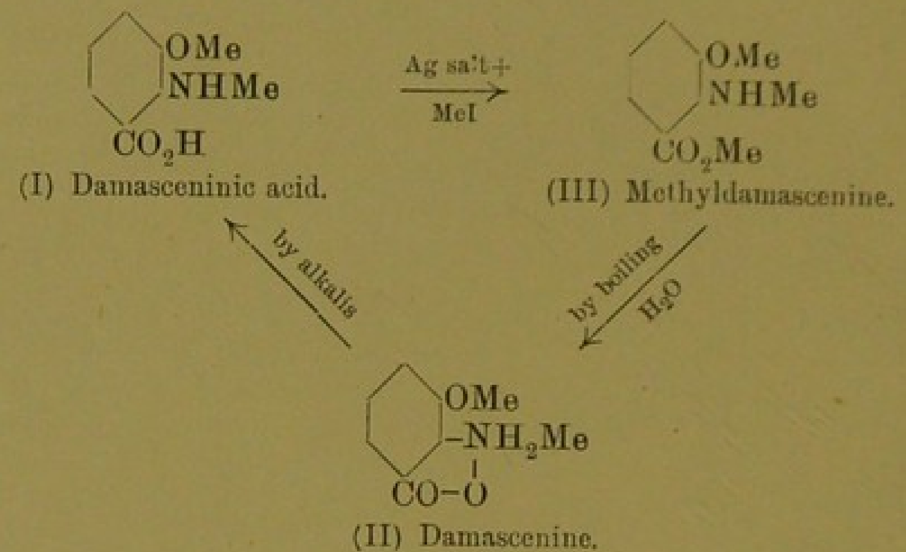
LXII.—*The Constitution and Synthesis of Damascenine, the Alkaloid of Nigella damascena.*

By Arthur James Ewins.

The alkaloid damascenine was first isolated by Schneider (*Pharm. Centr.-h.*, 1890, **31**, 173) from the seeds of *Nigella damascena.* He described it as a crystalline solid melting at 27°, and assigned to it the composition $C_{10}H_{15}O_3N$. This work is now shown to be substantially correct, the composition of the alkaloid being actually represented by the formula $C_{10}H_{13}O_3N$, differing only from the former in having two hydrogen atoms less. Until now, however, the work of Schneider has been obscured by the results of Pommerehne, who assigned to the alkaloid the formula $C_9H_{11}O_3N$ (*Arch. Pharm.*, 1900, **238**, 531), and stated that it was readily converted by alkalis into an isomeric acid, damasceninic acid. This formula was, moreover, accepted by Keller (*Arch. Pharm.*, 1904, **242**, 299), who later (*ibid.*, 1908, **246**, 1) obtained an alkaloid from *Nigella aristata* having the composition $C_{10}H_{13}O_3N$, which he termed "methyldamascenine." As will be shown later, however, this is the only alkaloid present in *Nigella*, and is identical with Schneider's damascenine.

The constitution of damasceninic acid, which is readily formed from damascenine, was, however, fairly conclusively shown by Keller to be 2-methylamino-3-methoxybenzoic acid (I), the synthesis of which he unsuccessfully attempted. From this acid "methyldamascenine" (III) was obtainable, but in order to explain the supposed isomerism of the acid with damascenine a betaine-like structure (II) was suggested, which involves a quinquevalent nitrogen atom linked in a manner which is without analogy, and is, as will be shown below, also without experimental foundation. The suggested relationship for the supposed three compounds is shown by the following scheme:



(I) Damasceninic acid.

(III) Methyldamascenine.

(II) Damascenine.

It was the doubt attaching to Keller's "betaine" formula which first suggested the present investigation. Since damasceninic acid could be converted into damascenine, the synthesis of the former was first attempted. Keller, starting from 3-nitromethylanthranilic acid, failed to achieve the synthesis; but it was accomplished with very little difficulty in the present instance by starting from *m*-hydroxybenzoic acid. The successive reactions employed will be referred to later. It was then somewhat unexpectedly found that the methyl ester of damasceninic acid was in all respects identical with the alkaloid damascenine as described by Schneider, and found by the author to be the only alkaloid obtainable from *Nigella damascena.*

In view of this result it was only necessary to explain some of the results obtained by Pommerehne and by Keller. The explanation becomes obvious if it is borne in mind that damascenine, now shown to be the methyl ester of an amino-acid, is very readily hydrolysed. Pommerehne himself (*Arch. Pharm.*, 1899, **237**, 475) emphasised the similarity of damascenine to the ester of anthranilic acid. Nevertheless, in the preparation of the hydrochloride of the alkaloid he evaporated a hydrochloric acid solution of the base at temperatures as high as 80° to 90°. The salt which he thus obtained, and which both he and Keller considered to be the hydrochloride of damascenine, was in reality a mixture consisting largely of the hydrochloride of damasceninic acid, and their conclusion that both damascenine and damasceninic acid had the same formula is consequently not surprising. That this is the explanation of many of their observations is clear when we consider some of these more closely; for example, Pommerehne was able to obtain only a small quantity (about 20 per cent.) of the theoretical amount of base from his hydrochloride. The numerous analyses of this salt, too, are by no means concordant. The difficulty of separation of such a mixture, too, is emphasised by Keller himself (*Arch. Pharm.*, 1908, **246**, 1), who by very careful fractionation of a hydrochloride obtained from *Nigella aristata* succeeded in obtaining a small quantity of the hydrochloride of the unchanged alkaloid (which he erroneously considered to be "methyldamascenine"), the greater part of the alkaloid having undergone hydrolysis, and yielded the impure mixture first obtained by Pommerehne. Further, Keller found that methyl iodide, acetic anhydride, acetyl chloride, and nitrous acid produced identical products from both damasceninic acid and the so-called damascenine hydrochloride, a result which he considered to be due to intramolecular rearrangement brought about by these reagents.

The identity of the two substances might have been suspected from the similarity in melting points of a number of salts of the two substances which are included in the following table, which also shows the identity of the synthetic compounds obtained in this investigation with those of the natural alkaloid described by Schneider or by the present author:

	Damascenine.				Damasceninic acid.			
	Natural alkaloid. (Schneider)	"Methyl-damascenine." (Keller)	Natural alkaloid. (Ewins)	Synthetic alkaloid. (Ewins)	Damasceninic acid. (Pommerehne)	"Damascenine." (Pommerehne)	Damascenic acid (natural). (Ewins)	Synthetic. (Ewins)
Base	27°	—	24–26°	23–24°	78° * 140–141° †	— —	— —	72–77° 141–142°
Hydrochloride (anhydrous)	—	—	156°	156°	209–210°	—	210–211°	210–211°
Hydrochloride + H_2O	121°	121°	122°	122°	—	197°		
Sulphate	168–170°	—	168–170°	168–169°	209–210°	203–205°	—	
Nitrate	98°	—	94–95°	94–95°	—	180°	—	
Hydrobromide	—	—	—	—	204–206°	197–199°	—	
Picrate	—	—	159°	159°	190°	189–190°	190°	190°
Platinichloride	165° ?	198°	193–194°	194°	202–203°	198°	201–202°	202°

* Acid + $3H_2O$. † Acid (anhydrous).

Synthesis of Damascenine.—This was accomplished by the following series of reactions. *m*-Hydroxybenzoic acid was converted into *m*-methoxybenzoic acid (m. p. 108°) by methyl sulphate and potassium hydroxide, the yield being practically quantitative. This acid on nitration under suitable conditions gave a mixture of nitro-derivatives, from which the required 2-nitro-3-methoxybenzoic acid was isolated without difficulty. This on reduction yielded 2-amino-3-methoxybenzoic acid, which on treatment with methyl iodide yielded the hydriodide of 2-methylamino-3-methoxybenzoic acid. This salt was converted into the corresponding hydrochloride, which was found to be identical with the hydrochloride of damasceninic acid. Keller's suggested constitution for the latter was thus confirmed. The acid on esterifying by Fischer's method gave a methyl ester, which proved to be identical with the natural alkaloid damascenine.

The steps in the synthesis are shown by the following scheme:

m-Hydroxybenzoic acid.
↓
m-Methoxybenzoic acid.
↓
2-Nitro-3-methoxybenzoic acid.
↓
2-Amino-3-methoxybenzoic acid.
↓
2-Methylamino-3-methoxybenzoic acid.
↓
Methyl 2-methylamino-3-methoxybenzoate (damascenine).

EXPERIMENTAL.

(*a*) *Preparation of Damascenine from the Seeds of* Nigella damascena.

Three kilograms of the finely ground seeds were extracted with light petroleum by shaking for some time and then filtering, and finally pressing out through fine muslin. The process was repeated until the extract on shaking with dilute acid gave a solution which no longer showed alkaloidal reactions. The petroleum extracts were combined and extracted with a 5 per cent. solution of hydrochloric acid. It is not advisable to concentrate the petroleum extract to any great extent, as in that case on shaking with dilute acid a troublesome emulsion is produced, and separation is effected only with difficulty. By directly extracting as described above, this difficulty is avoided. When all the alkaloid had been removed from the petroleum solution, the acid liquid was rendered alkaline by addition of solid sodium carbonate (the temperature being kept low by cooling in ice-water), and the oily precipitate which then formed extracted by shaking with ether. The ethereal solution was washed with water, dried, filtered, the solvent removed, and the yellow, oily residue distilled under diminished pressure. It distilled almost completely, and boiled constantly at 154°/15 mm. The pale yellow, oily liquid thus obtained was placed in a freezing mixture, when it solidified to a crystalline mass, which melted at 24—26°. Yield, 9·5 grams, or 0·32 per cent. of the seeds employed:

0·1092 gave 0·2466 CO_2 and 0·0660 H_2O. C=61·6; H=6·7.
0·1258 „ 8·8 c.c. N_2 (moist) at 19° and 740 mm. N=7·8.
$C_{10}H_{13}O_3N$ requires C=61·5; H=6·6; N=7·2 per cent.

The alkaloid boils at about 270°/750 mm. with slight decomposition. Its solutions in organic solvents show a very beautiful, blue fluorescence. The alkaloid so obtained agreed completely with the description of the base as given originally by Schneider (*loc. cit.*).

(*b*) *Synthesis of Damascenine.*

Methylation of m-*Hydroxybenzoic Acid.*—Fifty grams of *m*-hydroxybenzoic acid were dissolved in 200 c.c. of methyl alcohol, and a solution of 30 grams of sodium hydroxide in a minimum quantity of water was added. The mixture was then treated with 90 grams of methyl sulphate, when a vigorous reaction ensued. Potassium hydroxide in 50 per cent. aqueous solution was added in small quantities until the reaction became less vigorous. Sixty grams of methyl sulphate were then added in small quantities alternately with the solution of potassium hydroxide. The liquid was set aside for an hour, diluted with four volumes of water, filtered if necessary, and acidified with concentrated hydrochloric acid. The precipitated acid was collected at the end of an hour, and consisted of nearly pure *m*-methoxybenzoic acid melting at 107—108°. The yield was 45·5 grams, or 85 per cent. of the theoretical.

Nitration of m-*Methoxybenzoic Acid.*—To 20 grams of *m*-methoxybenzoic acid were added 80 c.c. of nitric acid (D 1·4). The mixture gradually became warm, the acid slowly dissolved, but almost immediately the mixture of nitro-derivatives began to separate. The temperature of the reaction mixture was never allowed to rise above 60°. Neglect of this precaution leads to a very much more vigorous reaction, and the yield of the required acid is greatly diminished. At the end of about three hours the mixture was diluted with water, the solid collected, and the crude product extracted with a small quantity of boiling alcohol. The less soluble residue was recrystallised from a larger volume of hot absolute alcohol. On cooling, 2-nitro-3-methoxybenzoic acid separated in rhombic plates melting at 249—250°. The acid was identified by analysis (Found, C=48·9; H=3·8; N=7·3. Calc., C=48·7; H=3·5; N=7·1 per cent.), melting point, and general properties with the acid obtained by Rieche (*Ber.*, 1889, **22**, 2352) from 2-nitro-3-methoxybenzaldehyde on oxidation with potassium permanganate.

Reduction of 2-Nitro-3-methoxybenzoic Acid to 2-Amino-3-methoxybenzoic Acid.—Ten grams of 2-nitro-3-methoxybenzoic acid were dissolved in 100 c.c. of absolute alcohol; 18 grams of tin foil and 60 c.c. of concentrated hydrochloric acid were then added alternately in small quantities, and the mixture was finally heated on the water-bath for three hours. The resulting solution was diluted with water, filtered, and the tin removed as sulphide. The filtrate was evaporated to small volume (40 c.c.), and solid sodium carbonate carefully added until the acid reaction to Congo-red just disappeared. After some time the crystalline *2-amino-3-methoxybenzoic acid* separated. It was recrystallised from water, and was then obtained in glistening, feathery clusters of thin plates melting at 162—163°. The yield was 5·15 grams, or 60 per cent. of the theoretical:

0·0744 gave 0·1572 CO_2 and 0·0362 H_2O. C=57·6; H=5·4.
0·1197 „ 8·8 c.c. N_2 (moist) at 19° and 761 mm. N=8·5.
$C_8H_9O_3N$ requires C=57·5; H=5·4; N=8·4 per cent.

2-Amino-3-methoxybenzoic acid is a glistening, crystalline solid, very sparingly soluble in cold, fairly readily so in hot, water. It is readily soluble in alcohol, ethyl acetate, or ether. It may be crystallised from hot benzene, from which it separates on cooling in masses of feathery needles. The solutions of the acid in organic solvents show a well marked blue fluorescence.

The *hydrochloride* is readily obtained by dissolving the acid in hot concentrated hydrochloric acid. On cooling, the hydrochloride separates in glistening, rhombic plates, melting at 204—205°:

0·1100 gave 0·0778 AgCl. Cl=17·5.
$C_8H_9O_3N,HCl$ requires Cl=17·4 per cent.

The *picrate* is readily obtained by treating a solution of the hydrochloride in water with a slight excess of a saturated aqueous solution of picric acid. It separates in deep red-coloured needles, melting at 172° to a red liquid:

0·1264 gave 0·1970 CO_2 and 0·0346 H_2O. C=42·5; H=3·0.
$C_8H_9O_3N,C_6H_3O_7N_3$ requires C=42·4; H=3·0 per cent.

The salt is sparingly soluble in cold, but moderately so in hot, water.

Methylation of 2-Amino-3-methoxybenzoic Acid.—Five grams of 2-amino-3-methoxybenzoic acid were dissolved in 25 c.c. of methyl alcohol, and 4·3 grams (1 molecular proportion) of methyl iodide added. The mixture was heated in a sealed tube for three hours at 100°. The liquid was then evaporated to dryness, the residue dissolved in a little water, and filtered from a small amount of insoluble material. The solution was then digested on the water-bath for an hour with an excess of freshly prepared moist silver chloride. The silver halides were removed by filtration, the filtrate evaporated to dryness, and the residue crystallised from absolute alcohol. There were thus obtained 4·25 grams of the hydrochloride of 2-methylamino-3-methoxybenzoic acid (damasceninic acid), melting sharply at 210—211°:

0·1418 gave 0·2559 CO_2 and 0·0743 H_2O. C=49·2; H=5·8.
0·1754 „ 0·1146 AgCl. Cl=16·2.
$C_9H_{11}O_3N$ requires C=49·6; H=5·5; Cl=16·3 per cent.

The identity of this salt with damasceninic acid hydrochloride was established by the following facts. The melting point of a mixture of equal weights of the synthetic and natural salts showed no lowering of the original temperatures, which were the same in each case. The platinichlorides (m. p. 202—203°) and picrates (m. p. 190°) obtained from both were identical. Further, by dissolving the synthetic hydrochloride in a little water, adding a calculated quantity of sodium carbonate sufficient to neutralise the hydrochloric acid contained in the salt, evaporating to dryness, and extracting with alcohol, a solid was obtained which crystallised from a concentrated aqueous solution and melted somewhat indefinitely from 72° to 77°. The dry solid on crystallisation from a mixture of chloroform and alcohol yielded stout, transparent, rhombic plates, melting at 141—142°, which were very hygroscopic. These properties are in complete agreement with those described by Keller for free damasceninic acid.

Methyl 2-Methylamino-3-methoxybenzoate (*Damascenine*).—Four grams of damasceninic acid hydrochloride were dissolved in 50 c.c. of methyl alcohol, the solution saturated with dry hydrogen chloride, and boiled under a reflux condenser for five hours. The solution was then concentrated and kept for some time, when 0·6 gram of a hydrochloride melting at 210—211° separated. This was collected, the filtrate evaporated to dryness, dissolved in a few c.c. of absolute alcohol, and dry ether added until crystallisation commenced. In this way a further quantity of a salt (1·4 grams) was obtained, melting at 201—202°. Although these salts were apparently almost pure damasceninic acid hydrochloride, both specimens when dissolved in water and the solution rendered alkaline with sodium carbonate were found to be mixed with a certain amount of the hydrochlorides of the methyl ester, as shown by the fact that in both cases a milky appearance was produced, due to the separation of the free ester. This was very marked in the second specimen.

The mother liquors from the crystalline product obtained above were evaporated to dryness and dissolved in a little cold water. The solution was rendered alkaline with sodium carbonate, and the oily precipitate extracted with ether. The ethereal solution (which showed a strongly marked, blue fluorescence) was washed with water until the washings were neutral, and then dried. On evaporating off the ether a residual oil was obtained, which distilled completely, boiling at 156—157°/17 mm. When placed in a freezing mixture the liquid solidified to a crystalline mass, which when pressed between filter paper at about 0° was found to melt at 23—24°:

0·1256 gave 0·2830 CO_2 and 0·0756 H_2O. C = 61·4; H = 6·7.

$C_{10}H_{13}O_3N$ requires C = 61·5; H = 6·6 per cent.

The identity of this synthetic ester with the naturally occurring damascenine was shown by the following facts. The boiling points and melting points of the two compounds are identical. A mixture of the two specimens melted at 23—25°, thus showing no lowering of melting point. Further, the salts obtained from both compounds were in all cases identical with each other and in good agreement with those originally described by Schneider, as shown in the table on p. 546.

Solutions of both compounds gave with mercuric chloride an oily precipitate, which crystallised within a few minutes, as also described by Schneider.

Two new salts of damascenine were obtained during this investigation, namely, the anhydrous hydrochloride and the picrate.

Damascenine Hydrochloride (*Anhydrous*).—This salt was readily prepared by precipitating a dry ethereal solution of the base by careful addition of an alcoholic solution of hydrogen chloride. It crystallises in slender prisms melting at 156°:

0·1490 gave 0·0920 AgCl. Cl = 15·27.

$C_{10}H_{13}O_3N,HCl$ requires 15·33 per cent.

The salt is deliquescent, and when crystallised from 80 per cent. alcoholic solution separates with one molecule of water of crystallisation, then melting at 121—122° as described by Schneider (*loc. cit.*) and by Keller (as "methyldamascenine" hydrochloride). The latter salt does not lose its water of crystallisation when dried in a vacuum over sulphuric acid, but the anhydrous salt may be obtained by carefully heating at 100°.

Damascenine picrate was obtained by precipitating an aqueous solution of a salt of the base with a saturated solution of picric acid in water. It crystallises in lemon-yellow, rhombic plates, melting at 158—159°, and, unlike damasceninic acid picrate, melts without the formation of a blue liquid:

0·1000 gave 0·1664 CO_2 and 0·0332 H_2O. $C=45·4$; $H=3·7$.
$C_{10}H_{13}O_3N,C_6H_3O_7N_3$ requires $C=45·3$; $H=3·8$ per cent.

The author wishes to express his thanks to Dr. G. Barger, by whom his attention was first drawn to the question dealt with in this investigation.

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