SOME OXIDATION REACTIONS OF DELCOSINE[1]

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ABSTRACT

The alkaloid delcosine was oxidized by chromic acid in acetic acid, and also by the Oppenauer reaction, to dehydrodelcosine in which the carbonyl was shown by infrared absorption to be in a pentatomic ring. Oxidation of the alkaloid with silver oxide gave two products: (*a*) N-desethyldelcosine, which could be N-acetylated or converted back to the original base by ethylation, thus proving the presence of an N-ethyl group; (*b*) a compound, $C_{24}H_{37}O_7N$, the properties of which agreed best with those of an internal ether, i.e., anhydrohydroxydelcosine. The action of N-bromosuccinimide on the alkaloid produced the same two compounds as silver oxide, plus a derivative, $C_{22}H_{33}O_7N$, that proved to be N-desethyl-anhydrohydroxydelcosine. Potassium permanganate oxidized delcosine to a product, $C_{22}H_{31}O_7N$, that had lost the N-ethyl group, contained the internal ether, and also a carbonyl in a five-membered ring. This same product was obtained on similar oxidation of N-desethyl-anhydrohydroxydelcosine. Oxidation of delcosine with mercuric acetate gave N-desethyl-delcosine and N-desethyl-anhydrohydroxydelcosine, together with a compound that was very soluble in water and proved to be the carbinolamine formed by hydroxylation of the methylene in the N-ethyl group. These results are discussed in terms of a structure that is tentatively advanced for delcosine.

Delcosine is a monoacidic base with an empirical formula that has been previously established as $C_{24}H_{39}O_7N$ (12). Of its seven oxygens three are known to be present in methoxyl groups and four in hydroxyls. Two of the hydroxyls are readily acetylated, but the remaining two are not (12), and are probably tertiary alcoholic groups. It does not take up any hydrogen in the presence of Adams' catalyst, thus indicating the absence of unsaturation. The ultraviolet spectrum of the bases, like that of lycoctonine (4), shows only end absorption and is similar to that of sparteine, which contains no unsaturation. As with lycoctonine, the end absorption is due to the basic nitrogen, with some contribution from hydroxyls and methoxyls (4). The infrared spectrum of delcosine does not contain any absorption band characteristic of a double bond. Delcosine must, therefore, possess a structure made up of six rings and, further, it has been shown by Goodson (7) probably to contain an N-ethyl group.

The behavior of delcosine towards various oxidizing agents has been investigated, and the results together with their interpretation in terms of a tentative structure are now reported.

Oxidation of delcosine with chromic acid in acetic acid or in dilute sulphuric acid gave a ketone $C_{24}H_{37}O_7N$ (I) in poor yield. The ketone formed mixed crystals with delcosine, but could be separated by paper chromatography or by countercurrent distribution. Its infrared spectrum (band at 1755 cm.$^{-1}$, no C—H stretching absorption at 2600–2800 cm.$^{-1}$) revealed it to be a five-membered ketone. It formed a monoöxime showing no carbonyl absorption in the infrared. Hence one of the hydroxyls in delcosine must be secondary and present in a five-membered ring. Attempts to condense the ketone I (dehydrodelcosine) with piperonal failed and, hence, it is probable that no methylene groups are present adjacent to the carbonyl. The same dehydrodelcosine was obtained by Oppenauer oxidation but the yield was also low and attempts to improve it by varying the conditions (cf. 13) proved fruitless.

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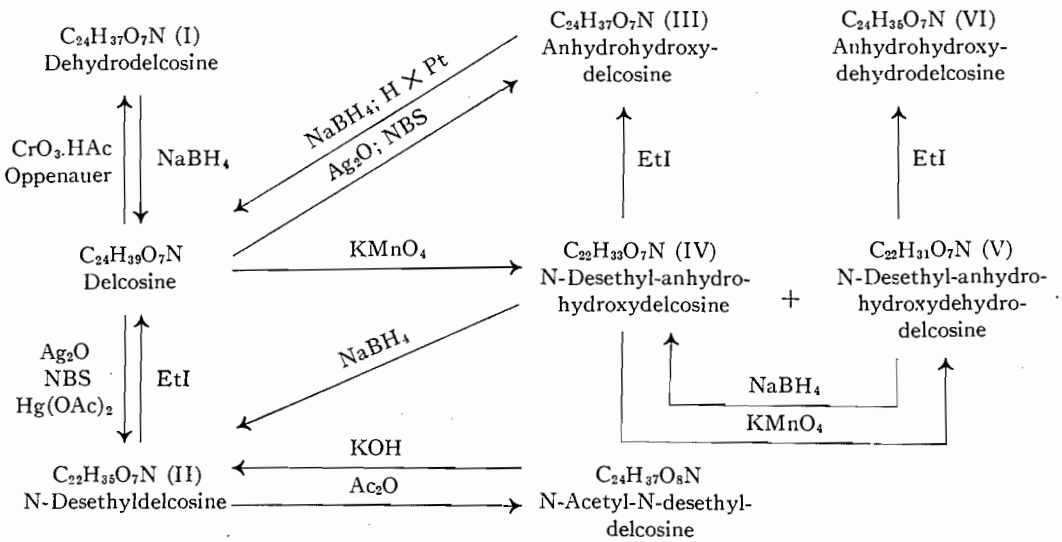
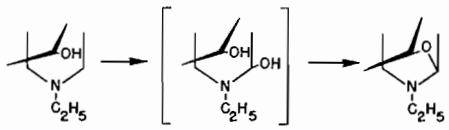
Delcosine was found to be inert to silver oxide under conditions which gave hydroxylycoctonine from lycoctonine (4). If, however, the reaction was carried out at 80° in ethanol for 68 hours, two products were obtained. One of these, $C_{22}H_{35}O_7N$ (II), was sparingly soluble in benzene; it contained five active hydrogens and was a stronger base (pK_a 7.9) than delcosine (pK_a 6.49). With acetic anhydride and pyridine it formed a neutral acetyl derivative showing an amide carbonyl absorption peak at 1643 cm.$^{-1}$ in the infrared. The acetyl derivative on hydrolysis gave back the basic product II, which must therefore be a secondary base. The base II was shown to be N-desethyldelcosine by treatment with ethyl iodide in dimethylformamide, which converted it to delcosine, thus firmly establishing the presence of an N-ethyl group in the alkaloid.



The second product of the silver oxide oxidation, $C_{24}H_{37}O_7N$ (III), was a much weaker base than the first (pK_a 4.2). Its infrared absorption spectrum showed no carbonyl nor double bond absorption, but contained sharp peaks at 1000 and 900 cm.$^{-1}$ which were absent in the spectrum of delcosine. As the pK_a of III showed a sharp drop of 2.29 units from that of delcosine, a change in the environment of the nitrogen such as the formation of a carbinolamine was suspected. (Lycoctonine on conversion to hydroxylycoctonine shows a drop in pK_a of three units.) The analysis, however, which showed that III contained the same number of oxygens as delcosine, and the fact that the compound formed a perchlorate that was not an anhydronium salt, provided evidence that compound III was not a simple carbinolamine.

If, on the other hand, it were assumed that one of the hydroxyls in the molecules was placed suitably to react with the carbinolamine formed initially, there could then arise an internal ether as indicated in the following partial formulae, and this would be in agreement with the pK_a of the compound and its analytical figures. In fact, compound III contained only three active hydrogens, thus substantiating the assumption. Cyclic carbinolamine internal ethers are not unknown in the field of alkaloids (cf. 11).

Reduction of compound III with sodium borohydride gave back delcosine, thus showing that no change had taken place in the stereochemistry of the molecule. Although



compound III was inert to hydrogenation in neutral medium, it rapidly took up 1 mole of hydrogen in aqueous perchloric acid in the presence of Adams' catalyst to give delcosine. There is thus no doubt that the net result of the oxidation was the abstraction of two hydrogens. Hence the reaction can be rationalized only on the basis of an internal ether formed from an intermediate carbinolamine.[3] Thus compound III is anhydrohydroxydelcosine (see partial formulae above).

N-Bromosuccinimide is known to oxidize secondary alcohols to ketones (6), and has been used in such a reaction by Cookson and Trevett (2) in their study of delpheline. Delcosine when oxidized with this reagent in buffered solution gave three products. One was identical with anhydrohydroxydelcosine (III) described above. The second product, $C_{22}H_{33}O_7N$ (IV), had a pK_a 4.6 and contained four active hydrogens. On treatment with ethyl iodide in dimethyl formamide it gave rise to III. Hence compound IV was clearly the internal ether of N-desethyldelcosine, i.e., N-desethyl-anhydrohydroxydelcosine. Reduction of IV with sodium borohydride gave N-desethyldelcosine (II), which was also obtained as the third product of the oxidation. The oxidation with N-bromosuccinimide was thus not specific, but attacked two carbon atoms vicinal to the nitrogen.

Oxidation of delcosine with potassium permanganate in acetone gave intractable material and some neutral product which could not be purified. On the other hand, potassium permanganate in N-potassium hydroxide at room temperature oxidized delcosine to two products. One was N-desethyl-anhydrohydroxydelcosine IV also obtained in the oxidation of delcosine with N-bromosuccinimide. The other was a high melting compound, $C_{22}H_{31}O_7N$ (V), the infrared absorption spectrum of which in nujol contained a band at 1741 cm.$^{-1}$ which shifted in chloroform solution to 1751 cm.$^{-1}$ indicating the presence of a five-membered cyclic ketone. In addition, the spectrum in nujol contained bands at 1000 and 900 cm.$^{-1}$ which were present in the spectra of III and IV, but absent in the spectra of delcosine, dehydrodelcosine (I), and N-desethyldelcosine, and can be considered as characteristic of the internal ether. Brief treatment with sodium borohydride reduced compound V quantitatively to N-desethyl-anhydrohydroxydelcosine IV. Hence the compound V was N-desethyl-anhydrohydroxy-dehydrodelcosine.[4]

N-Desethyl-anhydrohydroxydelcosine IV could be oxidized in over 60% yield to the corresponding ketone V. This increased yield indicated that in the oxidation of delcosine to the ketonic compound V, the formation of IV is the controlling factor. This oxidation is significant since secondary alicyclic alcohols do not give ketones in good yield under these conditions. The relatively high yield obtained may indicate that the secondary hydroxyl involved in the reaction is flanked on either side by quaternary carbons or, alternatively, by carbons located at bridgeheads, so that the ketone once formed cannot enolize.

The N-desethyl ketone V when ethylated with ethyl iodide gave rise to a new ketone,

[3]*It is possible to explain the analysis and the active hydrogen on the basis of hemiketal structure but the basic strength of compound III is incompatible with this. Furthermore, the infrared spectrum of the compound does not contain the absorption bands characteristic of such a structure (1), which is, therefore, very unlikely.*

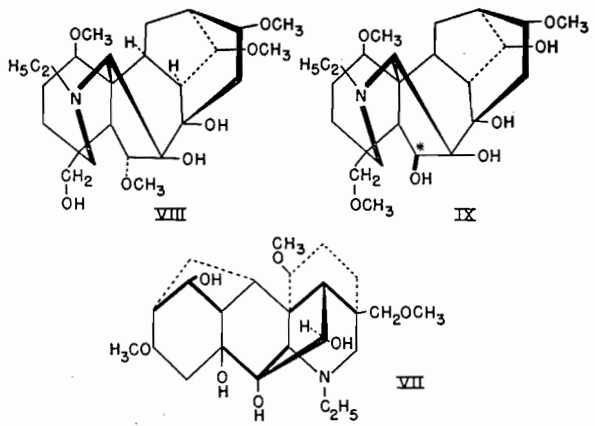
[4]*The high melting point of V and the shift in carbonyl frequency in its infrared spectrum may be due to considerable hydrogen bonding. In fact, the infrared showed a strongly bonded* NH.

$C_{24}H_{35}O_7N$ (VI) (infrared band at 1752 cm.$^{-1}$), still containing the internal ether (infrared bands at 1005 and 900 cm.$^{-1}$), which, therefore, must be anhydrohydroxy-dehydrodelcosine. Compound VI proved to be identical with a product obtained in minute quantity in the oxidation of delcosine with chromium trioxide – pyridine complex (9). It tended to form a hydrate and was difficult to extract.

In order to investigate whether the hydroxyl group oxidized by chromic acid or by the Oppenauer oxidation to give the ketone I was the same as that involved in the formation of compound V in the oxidation of IV, attempts were made to reduce selectively the ether system. For this purpose the ethylated derivative VI was used, but all attempts proved unsuccessful. An indication that the hydroxyl involved in both cases was the same can be obtained by a comparison of the pK_a values of the various compounds. The decrease in pK_a in going from delcosine (pK_a 6.49) to dehydrodelcosine I (pK_a 5.75) and from N-desethyl-anhydrohydroxydelcosine (IV) (pK_a 4.6) to the corresponding ketone V (pK_a 4.0) is small in both cases, i.e., 0.74 and 0.6 units respectively. The hydroxyl, therefore, does not seem to have been in close proximity to the nitrogen since Cookson and Trevett (3) have shown that in delpheline (pK_a 7.6) oxidation of a hydroxyl close to the nitrogen to give dehydrodelpheline (pK_a 5.5) caused a decrease in pK_a of 2.1 units.

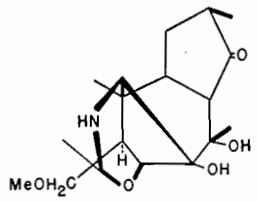
Silver acetate oxidation of delcosine gave anhydrohydroxydelcosine III together with some N-desethyldelcosine (II). Oxidation with mercuric acetate in acetic acid gave a mixture of N-desethyl-anhydrohydroxydelcosine (IV), N-desethyldelcosine (II), and a compound which was very soluble in water and was not isolated. The aqueous solution containing this compound was acidified and some of the water distilled off. The distillate contained acetaldehyde which was isolated as its *p*-nitrophenylhydrazone. The remaining acid aqueous solution was rendered basic with ammonia and on extraction yielded a further quantity of N-desethyldelcosine II. The water-soluble compound which could not be isolated as such must, therefore, have been the carbinolamine of delcosine formed by hydroxylation of the methylene in the ethyl group which was subsequently split by acid to N-desethyldelcosine and acetaldehyde. A similar oxidation in delpheline was observed by Cookson and Trevett (3).

Delcosine ($C_{24}H_{39}O_7N$), lycoctonine ($C_{25}H_{41}O_7N$), and delpheline ($C_{25}H_{39}O_6N$) are three *Delphinium* alkaloids, and the first two occur together in some species. If their substituents be replaced by hydrogen, all three can be represented by the common saturated empirical formula $C_{21}H_{33}N$, which is that of a hexacyclic structure. It is, therefore, legitimate to assume that the three alkaloids possess closely related structures.



The chemical investigation of lycoctonine has led to a partial elucidation of its structure (5) while an X-ray crystallographic analysis has made it possible to advance a total structure VII (10) which can also be written in the diterpenoid form VIII.[5] In order to accommodate the chemistry of delpheline which parallels that of lycoctonine, Cookson and Trevett (3) have recently suggested the same ring structure for delpheline in which, however, some of the substituents are different although similarly located. The behavior of delcosine towards oxidizing agents described above and the acylation results described previously (12) can be rationalized by assuming for delcosine a structure IX with the same ring arrangement as that proposed for lycoctonine. The difference between delcosine, lycoctonine, and delpheline would then rest with the oxygenated substituents and with the different orientation of the substituent at the asterisked position in IX.

To account for the formation of N-desethyl-anhydrohydroxy-dehydrodelcosine V, which contains both an internal ether and a five-membered ketone, it is necessary that the substituent on the five-membered ring remote from the nitrogen should be a secondary hydroxyl. Also that a hydroxyl be present in the pentatomic ring adjacent to the heterocyclic ring instead of the methoxyl present in lycoctonine, and that its orientation be opposite to that obtaining in lycoctonine. It is this particular orientation of the hydroxyl that accounts for the formation of an internal ether instead of a lactam. Lycoctonine (4) and delpheline (2) both form lactams on oxidation, and the latter contains a hydroxyl in the same position as that marked with an asterisk in IX. This hydroxyl in delpheline just like the methoxyl in lycoctonine must, therefore, be oriented away from the α-position to the nitrogen. On the basis of structure IX the compound V (N-desethyl-anhydrohydroxy-dehydrodelcosine) can be represented as shown by the partial formula V, which also shows the internal ether.



V

In the course of the isolation of the various oxidation products described, no acidic material was isolated, and it is concluded that no primary alcoholic group is present in the alkaloid. The presence of a vicinal triol system in delcosine should be detectable by oxidation with periodate, but this oxidation gave anomalous results, and up to 5 moles of oxidant were consumed in 120 hours. On the other hand, anhydrohydroxydelcosine (in which one of the hydroxyls is bound in an internal ether) on similar oxidation consumed 1 mole of periodate, thus indicating the presence of vicinal hydroxyls in that compound. The results of the periodate cleavage studies will be reported later.

Although the size of the heterocyclic ring, and many features paralleling lycoctonine chemistry still have to be proved, we tentatively propose to represent delcosine by structure IX on the basis of which all the known facts can be rationalized.

[5]*We feel that the representation VII of the structure of lycoctonine used heretofore by this group is a better illustration of the spatial model produced by X-ray crystallography than the diterpenoid representation. Further, the diterpenoid formula gives a distorted aspect to the rings, and does not lend itself as well to the illustration of the various rearrangements undergone by lycoctonine. Since, however, most of the workers in this field of alkaloid chemistry are using the diterpenoid representation, we are adopting it in spite of its shortcomings, in order to avoid confusion in the literature.*

EXPERIMENTAL

All the infrared spectra were measured in nujol mulls (unless otherwise mentioned) on a Perkin-Elmer double-beam spectrometer model 21B, using a sodium chloride prism. The specific rotations were measured in chloroform solutions (accuracy $\pm 1.5°$) and the pK_a were determined in 50% ethanol by electrometric titration.

Oxidation of Delcosine with Chromic Acid in Acetic Acid (Dehydrodelcosine I)

To a solution of delcosine (1.35 g.) in glacial acetic acid (10 ml.) chromium trioxide (0.505 g.) was added portionwise. The solution was set aside for 24 hours at room temperature, and then refluxed for 90 minutes. The solvent was removed under reduced pressure and the residue partitioned between dilute sulphuric acid and chloroform (100 ml.). The chloroform layer was washed with a fresh quantity of dilute acid, and the combined acid extract and washings brought to pH 10 with aqueous 40% sodium hydroxide. This alkaline solution was extracted with chloroform and the extract on evaporation left 0.926 g. of a gummy basic product. The chloroform layer separated from the dilute sulphuric acid solution yielded acidic and neutral material (0.139 g.) but no crystalline product could be obtained from this fraction.

The gummy basic product was dissolved in benzene and chromatographed on a column of grade V alumina. Elution with the first 100 ml. of benzene gave a gum, whereas the next 100 ml. gave a partially crystalline material (259 mg.). This was recrystallized from ethanol, m.p. 199–200°. Mixture with delcosine depressed its melting point. Its infrared absorption spectrum in nujol showed a band at 1755 $cm.^{-1}$. The further eluates from the chromatogram yielded unchanged delcosine.

Paper chromatography, by the descending technique on Whatman No. 1 paper buffered to pH 4.78 (8) with butanol saturated with water as solvent, showed that the crystalline product consisted of a mixture of delcosine (R_f 0.546) and a ketonic substance (R_f 0.725). The spots were made visible with Dragendorff's reagent.

In a second experiment, delcosine (6.75 g.) dissolved in acetic acid (50 ml.) was oxidized with chromic acid (2.525 g.) at room temperature. After the products were worked up as above, the acid fraction obtained weighed 1.07 g. It was amorphous, and chromatography on alumina or celite failed to yield a crystalline substance. On the other hand, the basic fraction (4.406 g.) when chromatographed on grade II alumina gave 1.23 g. of a mixture of gummy and crystalline material. This mixture when chromatographed again on alumina and eluted with 300 ml. of benzene containing 20% of chloroform yielded the ketonic product (0.357 g.). This was crystallized from a mixture of benzene and petroleum ether from which it separated as colorless prisms. Five recrystallizations from dilute ethanol furnished an analytical sample of dehydrodelcosine, m.p. 212.5–213.5°. Found: C, 63.58; H, 8.10. Calc. for $C_{24}H_{37}O_7N$: C, 63.83; H, 8.26%. The pK_a was 5.75 and the molecular weight 457 (calc. 451). The infrared absorption spectrum contained a strong band at 1755 $cm.^{-1}$. A paper chromatogram of the pure dehydrodelcosine gave one spot (R_f 0.725).

In a later experiment, it was found more convenient to purify the ketone by countercurrent distribution. The crude mixture of basic products (3.0 g.) was distributed in 12 transfers between benzene (100 ml. in each tube) and a buffer (100 ml.) pH 4.92, made by mixing 55 volumes of M dipotassium acid phosphate and 45 volumes of 0.5 M citric acid. When the distribution was complete, the aqueous solutions were made alkaline with aqueous 40% sodium hydroxide (13 ml.) and the organic solutes driven into

the benzene layers. Delcosine (1.52 g.) was contained in tubes 11 and 12 whereas dehydrodelcosine was found in tubes 7, 8, and 9 (568 mg.). No crystalline product was isolated from the other tubes although a peak occurred in tube 3.

Dehydrodelcosine Oxime

Hydroxylamine hydrochloride (58 mg.) and dehydrodelcosine (95 mg.) were dissolved in ethanol, and the pH of the solution brought to 9 by addition of 10% sodium hydroxide. After the solution had refluxed for 3.5 hours the solvent was evaporated under reduced pressure and the residue dissolved in water. The pH was adjusted to 7 and the solution extracted with chloroform. Evaporation of the chloroform extract gave the crystalline oxime, which, after recrystallization from ethanol, consisted of colorless needles, m.p. 229.5–230.5°. Found: C, 62.03; H, 8.44; N, 6.09. Calc. for $C_{24}H_{38}O_7N_2$: C, 61.78; H, 8.21; N, 6.00%. The infrared absorption spectrum contained no band in the carbonyl region.

Oppenauer Oxidation of Delcosine

A solution of aluminum isopropoxide (7.26 g.) in dry benzene (100 ml.) was added in the course of 30 minutes to a refluxing solution of delcosine (1.35 g.) in dry benzene (60 ml.) containing cyclohexane (40 ml.). The mixture was refluxed for 4 hours, left overnight at room temperature, and refluxed for a further 5 hours. It was acidified with glacial acetic acid and steam distilled to remove the volatile solvents. After cooling, the residual aqueous solution was brought to pH 1 with 3 *N* sulphuric acid and extracted with chloroform. The pH was then adjusted to 10 by the addition of 40% sodium hydroxide and the solution extracted with chloroform. This chloroform extract, on evaporation, left a gummy residue, 1.24 g., which was dissolved in ethanol. On cooling, delcosine (0.33 g.) crystallized. The residual basic material recovered from the mother liquor was dissolved in benzene and chromatographed on grade V alumina. The eluate yielded the mixture of delcosine and dehydrodelcosine, m.p. 199–200°, wt. 181 mg., previously described.

Reduction of Dehydrodelcosine

Dehydrodelcosine (100 mg.) and sodium borohydride (30 mg.) were added to methanol containing a few drops of water (3 ml.) and the mixture left at room temperature for 15 minutes. The solution was then heated on the steam bath for 1 hour, diluted, and allowed to cool. Colorless, glistening hexagons separated out, m.p. 201–203°, either alone or mixed with delcosine.

Silver Oxide Oxidation of Delcosine

Freshly prepared silver oxide (from 7.4 g. of silver nitrate) was added to a solution of delcosine (2.86 g.) in ethanol (150 ml.) and water (150 ml.). The mixture was kept at 80° with vigorous stirring for 68 hours, then cooled and filtered. The residue of metallic silver and silver oxide was washed well with ethanol and the combined filtrate and washings was evaporated to a sirup under reduced pressure. This sirup was partitioned between 3 *N* sulphuric acid and chloroform, and the aqueous layer separated and extracted with three 50 ml. portions of chloroform. The combined chloroform extract was back extracted with 3 *N* sulphuric acid, washed with water, and evaporated to dryness. It left a brown residue (30 mg.) which was not further investigated.

The combined acid liquor and washings was made alkaline with ammonia and extracted with chloroform. Evaporation of the chloroform extract gave a colorless residue (2.326 g.). This, on trituration with benzene (5 ml.), left a crystalline compound (0.679 g.), m.p. 230–232°, which, after recrystallization from ethanol (charcoal), melted at 240–241° (dec.) (N-desethyldelcosine II), pK_a 7.9, $[\alpha]_D^{22}$ +79.5° (*c*, 0.88). Found: C, 62.13; H, 8.24; N, 3.36; act. H (in dioxane), 1.05. Calc. for $C_{22}H_{35}O_7N$: C, 62.09; H, 8.29; N, 3.29; 5 act. H, 1.18%.

The benzene liquor left after filtration of N-desethyldelcosine was diluted with benzene and chromatographed on grade V alumina. It was eluted with benzene and the first 125 ml. of eluate contained a base, 0.602 g., m.p. 187–188°. Further elution with benzene (325 ml.) and then with benzene containing 1% ethanol (100 ml.) yielded a further 0.258 g. of the same product. Continued elution with benzene containing 1% ethanol (300 ml.) gave N-desethyldelcosine, 0.155 g., m.p. 240–241° (dec.).

The product melting at 187–188° (anhydrohydroxydelcosine III) had pK_a 4.2 and $[\alpha]_D^{22}$ +74.6° (*c*, 1.4). Found: C, 63.92; H, 8.04; N, 3.35; act. H, 0.56. Calc. for $C_{24}H_{37}O_7N$: C, 63.83; H, 8.26; N, 3.10; 3 act. H, 0.66%. Its infrared absorption spectrum showed no carbonyl nor double bond absorption but contained two sharp bands at 1000 and 900 cm.$^{-1}$.

Anhydrohydroxydelcosine Perchlorate

A solution of anhydrohydroxydelcosine (161 mg.) in methanol (5 ml.) was made just acid by the cautious addition of 70% perchloric acid. Dropwise addition of ethyl acetate with cooling and stirring caused the salt to separate as colorless needles (141 mg.). Recrystallized from methanol–ether containing a trace of perchloric acid, it melted at 216–217° (dec.). Found: C, 51.94; H, 7.07; N, 2.52. Calc. for $C_{24}H_{37}O_7N.HClO_4$: C, 51.90; H, 6.90; N, 2.52%. The infrared absorption spectrum of the salt contained no absorption bands in the region 1550–1800 cm.$^{-1}$. The perchlorate is therefore not an anhydronium salt.

Ethylation of N-Desethyldelcosine

N-Desethyldelcosine (35 mg.), sodium carbonate (39 mg.), and dimethylformamide (250 mg.) containing ethyl iodide (79 mg.) were heated on the steam bath for 6.5 hours. The solution was cooled, acidified with dilute sulphuric acid, and extracted with chloroform. It was then rendered basic with ammonia and again extracted with chloroform. This second extract, on evaporation, gave colorless rhombs, m.p. 197–202°, which on recrystallization from dilute methanol, separated as hexagons, m.p. 201–202°, either alone or mixed with delcosine.

Acetylation of N-Desethyldelcosine

A mixture of N-desethyldelcosine (141 mg.), pyridine (two drops), and acetic anhydride (2.5 ml.) was left at room temperature for 20 hours. A sufficient quantity (15 ml.) of aqueous sodium carbonate to produce a basic solution[6] was added. Extraction with chloroform gave a colorless gum (153 mg.) that could not be crystallized, and was possibly a mixture of O,N-diacetate and O,O,N-triacetate. It was hydrolyzed with aqueous sodium carbonate on the steam bath for 5 hours and, after cooling, the solution was extracted with chloroform. The extract, on evaporation, left a crystalline residue, m.p. 221–225°, which in admixture with N-desethyldelcosine melted at 190–220°.

[6]*It is important to keep the aqueous solutions basic throughout, otherwise the product cannot be crystallized.*

N-Acetyl-N-desethyldelcosine, after crystallization from acetone, consisted of colorless needles, m.p. 221–225°. Found: C, 61.78; H, 8.17; N, 3.07. Calc. for $C_{24}H_{37}O_8N$: C, 61.65; H, 7.98; N, 3.00%. The infrared absorption spectrum showed no band at 1730 cm.$^{-1}$ characteristic of an acetoxy group, but contained a band at 1643 cm.$^{-1}$ characteristic of an amide carbonyl.

Hydrolysis of N-Acetyl-N-desethyldelcosine

The N-acetyl derivative (50 mg.) was hydrolyzed by being heated on the steam bath with 20% aqueous potassium hydroxide for 2 hours. Extraction of the cooled solution with chloroform gave N-desethyldelcosine, m.p. 239–241°, either alone or mixed with an authentic sample.

Reduction of Anhydrohydroxydelcosine

(*a*) Anhydrohydroxydelcosine (258 mg.) was dissolved in 50% aqueous methanol (5 ml.), and sodium borohydride (50 mg.) was added to the solution. The solution was left standing at room temperature for 18 hours, and subsequently heated on the steam bath for 1 hour. After cooling, the solution was extracted with chloroform, and the extract, on evaporation, left a residual white powder, m.p. 196–200°, wt. 236 mg. This was purified by chromatography on alumina and crystallized from dilute methanol from which it separated as colorless hexagons, m.p. 201–203° either alone or mixed with delcosine.

(*b*) Anhydrohydroxydelcosine (54.6 mg.) was hydrogenated in aqueous perchloric acid in the presence of Adams' catalyst (33 mg.) at atmospheric pressure and room temperature (26°). One mole of hydrogen was absorbed smoothly in 30 minutes, but no further uptake of hydrogen was observed within the next 2 hours. The solution was diluted with water and the catalyst filtered and washed with dilute ethanol. The combined filtrate and washings was made alkaline with ammonia and extracted with chloroform. Evaporation of the extract left a crystalline residue (45 mg.), m.p. 199–201° and, in admixture with delcosine, m.p. 200–203°.

Oxidation of Delcosine with N-Bromosuccinimide

Delcosine (500 mg.) was dissolved in aqueous acetone (4 acetone:1 water, 10 ml.) containing a few drops of glacial acetic acid. Sodium acetate (450 mg.) and N-bromosuccinimide (1.75 equivalents) were added at room temperature. On addition of the latter the solution turned yellow, and the color disappeared in 30 seconds as some heat was evolved. The solution was diluted with water (30 ml.) and allowed to stand at room temperature for 20 hours. Dilute sulphuric acid was added to bring the solution to pH 2, and the acidic and neutral material was removed by extraction with chloroform. The aqueous solution was then made alkaline with ammonia and again extracted with chloroform. The basic product (350 mg.) left after evaporation of this chloroform extract was dissolved in benzene and chromatographed on grade V alumina. Elution with benzene–ether (1:1) gave anhydrohydroxydelcosine III (197 mg.), which crystallized from acetone–petroleum ether as colorless prisms, m.p. 188–189°, identical with the compound obtained from the silver oxide oxidation. Further elution with benzene containing 10% ethanol gave N-desethyldelcosine II (138 mg.), which after crystallization from ethanol melted at 239–240°. Mixture with N-desethyldelcosine obtained in the silver oxide oxidation failed to alter the melting point, and comparison of the infrared absorption spectra confirmed the identity.

In a second experiment, delcosine (578 mg.) was dissolved in acetone and oxidized with N-bromosuccinimide (358 mg.) in the presence of sodium carbonate (480 mg.). The solution (pH 8–9) was diluted with water and left at room temperature for 20 hours. When worked up as above, anhydrohydroxydelcosine (80 g.) was obtained. The N-desethyldelcosine fraction, however, was accompanied by a compound separating as needles which was very soluble in acetone. It was readily separated from N-desethyldelcosine, which is sparingly soluble in acetone. The new product crystallized from acetone–petroleum ether as needles, m.p. 218–219°, which proved to be N-desethyl-anhydrohydroxydelcosine IV. It had pK_a 4.6 and $[\alpha]_D^{22}$ +132° (c = 0.75). Found: C, 62.60, 62.47; H, 7.82, 7.97; N, 3.85. Calc. for $C_{22}H_{33}O_7N$: C, 62.39; H, 7.85; N, 3.31%.

Ethylation of N-Desethyl-anhydrohydroxydelcosine

N-Desethyl-anhydrohydroxydelcosine (70 mg.) was added to a solution of sodium carbonate in freshly distilled ethyl iodide (275 mg.) and dimethylformamide (350 mg.) and heated on the steam bath for 5 hours. The solution was cooled overnight, during which silky needles were deposited. The crystalline hydriodide was dissolved in water, and the solution made basic with sodium hydroxide and extracted with chloroform. The product obtained from the extract consisted of anhydrohydroxydelcosine (50 mg.), which after purification by chromatography melted at 187–188° either alone or in admixture with an authentic sample. Comparison of the infrared absorption spectra which were superimposable confirmed the identity.

Reduction of N-Desethyl-anhydrohydroxydelcosine

The compound (88 mg.) was dissolved in 50% aqueous methanol and sodium borohydride (23 mg.) was added to the solution which was kept at room temperature for 20 hours, subsequently heated on the steam bath for 10 minutes, and allowed to cool. On standing, crystals of N-desethyldelcosine (20 mg.) separated. Chloroform extraction of the mother liquor yielded a further 35 mg. of the same compound. After recrystallization the melting point was raised to 240–241° and was undepressed by mixing with an authentic sample of N-desethyldelcosine.

Oxidation of N-Desethyl-anhydrohydroxydelcosine with Potassium Permanganate

N-Desethyl-anhydrohydroxydelcosine (194 mg.) was dissolved in 5% aqueous potassium hydroxide (10 ml.) and a 1% solution of potassium permanganate in 5% aqueous potassium hydroxide (30 ml.) was added gradually over an hour at room temperature. The solution was then diluted and warmed on the steam bath to complete the reaction. The manganese dioxide was filtered off and the excess permanganate reduced with a concentrated solution of sodium sulphite. The liquor was then extracted with chloroform and the extract evaporated to dryness. There was left a crystalline residue (122 mg.), m.p. 275–293°. Repeated recrystallization from acetone–petroleum ether and from ether – petroleum ether raised the melting point to 298°. Its pK_a was 4.0. Found: C, 62.61; H, 7.99; N, 3.48. Calc. for $C_{22}H_{31}O_7N$: C, 62.69; H, 7.41; N, 3.32%. The analytical figures agree with those required by N-desethyl-anhydrohydroxy-dehydrodelcosine and the infrared absorption spectrum contained the two bands at 1000 cm.$^{-1}$ and 900 cm.$^{-1}$ typical of the internal ether and a sharp band at 1743 cm.$^{-1}$ indicating a five-membered cyclic ketone.

Reduction of N-Desethyl-anhydrohydroxy-dehydrodelcosine

The keto compound (12 mg.) was reduced with sodium borohydride by heating (12 mg.) in aqueous methanol on the steam bath for 1 hour. Chloroform extraction of

the reaction mixture gave the product (11 mg.) as needles, m.p. 213–215°. Recrystallized from acetone–petroleum ether, it consisted of colorless needles, m.p. 216–218°, either alone or in admixture with N-desethyl-anhydrohydroxydelcosine.

Ethylation of N-Desethyl-anhydrohydroxy-dehydrodelcosine

A mixture of N-desethyl-anhydrohydroxy-dehydrodelcosine (50 mg.), ethyl iodide (1 ml.), dimethylformamide (20 mg.), and sodium carbonate (20 mg.) was heated on the steam bath for 5 hours. The reaction mixture was evaporated to dryness, and the residue triturated with hot dry acetone and filtered. The filtrate was evaporated to dryness, the residue taken up in dry benzene containing a little acetone, and the solution filtered and evaporated to dryness. The residual anhydrohydroxy-dehydrodelcosine was crystallized from alcohol–ether from which it separated as needles, m.p. 192–193°. $[\alpha]_D^{23}$ +34.9° (c = 1.46). Found: C, 63.95; H, 7.95; N, 3.14. Calc. for $C_{24}H_{35}O_7N$: C, 64.12; H, 7.85; N, 3.12%.

Oxidation of Delcosine with Potassium Permanganate[7]

Delcosine (231 mg.) was dissolved in N potassium hydroxide and the solution cooled to 0°. A solution of 1% of potassium permanganate in N potassium hydroxide was added dropwise to the stirred solution of delcosine. The addition was continued until the permanganate color persisted and during the addition the temperature was allowed to rise to room temperature. The excess permanganate was destroyed with sodium sulphite, the solution filtered, and the filtrate extracted with chloroform. Evaporation of the extract left a crystallized residue (65 mg.), m.p. 210–280°. Fractional crystallization from a mixture of chloroform, ether, and petroleum ether gave a compound separating as needles (44 mg.), m.p. 280–285°, that proved to be identical with N-desethyl-anhydrohydroxy-dehydrodelcosine (see above), and also a smaller quantity (17 mg.) of N-desethyl-anhydrohydroxydelcosine, m.p. 218°.

Oxidation of Delcosine with Silver Acetate

Delcosine (405 mg.) and silver acetate (1.445 g.) were refluxed in 3% acetic acid (50 ml.) for 40 minutes. The hot solution was filtered from the metallic silver, and filtered again, after cooling, from the silver acetate that had crystallized. Dilute sulphuric acid was added to the filtrate and the acid solution extracted with chloroform. The aqueous solution was then made alkaline with ammonia and again extracted with chloroform. The gum left after evaporation of the chloroform was dissolved in benzene and chromatographed as usual. It yielded anhydrohydroxydelcosine (230 mg.), m.p. 187–188°, undepressed by mixture with the sample obtained by silver oxide oxidation. No other pure product could be isolated from the reaction.

Oxidation of Delcosine with Mercuric Acetate

Delcosine (3.0 g.) was dissolved in 3% acetic acid (65 ml.), and mercuric acetate (16.8 g.) was added. The solution was heated on the steam bath for 60 minutes. The precipitated mercurous acetate was filtered off and the filtrate extracted with chloroform. Evaporation of the chloroform extract left a mixture of N-desethyldelcosine and N-desethyl-anhydrohydroxydelcosine which was directly reduced with sodium borohydride to give N-desethyldelcosine (1.9 g.). The aqueous solution that had been extracted was acidified with dilute hydrochloric acid, and an aliquot (25 ml.) distilled at atmospheric pressure to about half volume. The aqueous distillate was treated with an acetic acid solution of p-nitrophenylhydrazine containing a few drops of hydrochloric acid. Yellowish

[7]*This oxidation was first carried out by Dr. W. E. Walles in this laboratory.*

brown needles separated, m.p. 127–130.5°. Recrystallization from aqueous methanol raised the melting point to 130–130.5°, and this was undepressed by mixing with an authentic sample of acetaldehyde *p*-nitrophenylhydrazone.

The acidic solution from which the aliquot had been taken was hydrolyzed on the steam bath, cooled, made alkaline with ammonia, and extracted with chloroform. The extract on evaporation yielded a further quantity of N-desethyldelcosine.

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