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The Insecticidal Principles of *Haplophyton cimicidum*. II. Haplophytine and Cimicidine[1]

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Several crystalline derivatives confirm the empirical formula $C_{23}H_{28}O_5N_2$ for cimicidine. One nitrogen atom is basic, and the compound does not react with cyanogen bromide or carbon tetrachloride. Two carbonyls probably are present. The alkaloid was slowly hydrolyzed by sulfurous acid to give unstable products. A carbon-to-carbon double bond is indicated by the ready absorption of one mole of hydrogen. Alkaline hydrogen peroxide added one atom of oxygen to cimicidine but the alkaloid was not readily attacked by permanganate. Haplophytine and cimicidine reacted with acetic anhydride to give water-soluble products. Both alkaloids were reduced by sodium borohydride to hydroxy compounds.

The empirical formula $C_{23}H_{28}O_5N_2$[4] has been confirmed for cimicidine by the preparation and analysis of a crystalline hydrochloride, picrate and chloroplatinate. The formation of monoacidic salts indicates the presence of only one basic nitrogen atom; the N-methyl determination was negative.[4] The absence of absorption in the range 3700–3000 cm.$^{-1}$ in the infrared and the fact that only salt formation was noted with acetyl chloride indicate that the nitrogen atoms are tertiary. The basic nitrogen atom of cimicidine, unlike that of haplophytine, did not react with cyanogen bromide or carbon tetrachloride.[5] Cimicidine appeared to form a methiodide with the simultaneous addition of one mole of iodine. The reason for the acidity of cimicidine, like that of haplophytine, is not yet understood.

Of the five oxygen atoms present in cimicidine, one is present as a methoxyl group[4] and at least two as carbonyl groups. The infrared absorption spectrum shows two strong absorption bands at 1751 and 1632 cm.$^{-1}$ and there is a definite shoulder at 1774 cm.$^{-1}$.[4]

Cimicidine was slowly hydrolyzed in sulfurous acid to give a light yellow, apparently crystalline, solid. However, the hydrolysis product was so unstable in air that all attempts to isolate it were unsuccessful.

Cimicidine quickly absorbed one mole of hydrogen per mole of alkaloid over platinum at room temperature and atmospheric pressure. Dihydrocimicidine was obtained as a dihydrate and formed a crystalline picrate and hydrochloride. It reacted with acetic anhydride to form a water-soluble derivative which, in turn, formed a hydrochloride. Alkaline hydrogen peroxide reacted with cimicidine but the reaction was different from that of haplophytine.[5] The product seemed to be $C_{23}H_{28}O_6N_2$ (addition of [O]) and it was almost non-basic. It dissolved in concentrated hydrochloric acid but was recovered unchanged on dilution with water. Neutral permanganate did not affect materially cimicidine at room temperature in one hour.

Both haplophytine and cimicidine reacted with acetic anhydride in pyridine to give water-soluble products. The product in the case of haplophytine appeared to result from the addition of the elements of acetic acid but with butyric and benzoic anhydrides the products were butyryl and benzoyl derivatives indicating that the acetylated product probably crystallizes with a molecule of water. The acetylated product of cimicidine was an acetyl derivative. Acetylated haplophytine has a comparatively low melting point (192–195°) and its infrared spectrum is noteworthy for the disappearance of the 1656 cm.$^{-1}$ band of haplophytine and the appearance of an absorption band at 1710 cm.$^{-1}$ and a shoulder at 1765 cm.$^{-1}$. On acetylation of cimicidine, the 1632 cm.$^{-1}$ band is replaced by one at 1671 cm.$^{-1}$. These acetylated products can be converted to the parent alkaloid by treatment with dilute alkali. Neither haplophytine nor cimicidine reacted with diazomethane and the only reaction with acetyl chloride was salt formation. The O–H and N–H region of the infrared spectra of both alkaloids showed no significant absorption. The ready reversibility of the acetylations and the apparent lack of acetylatable functions in both alkaloids make an interpretation of these reactions difficult. The function of haplophytine and cimicidine which is acetylated must either arise by isomerization of the alkaloids, which appears unlikely, or is an acetylatable group that shows no characteristic absorption in the infrared. *o*-Hydroxyacetophenone, for example, shows no absorption band in the O–H region of the infrared spectrum and acetylation causes a shift in the carbonyl frequency from 1635 to 1678 cm.$^{-1}$ with the appearance of an acetoxy band at 1762 cm.$^{-1}$.[6]

Treatment of haplophytine and cimicidine with sodium borohydride gave highly hygroscopic reduction products. In both cases the infrared spectra showed the loss of the 1751 cm.$^{-1}$ absorption band with the appearance of a band in the region of the spectrum indicative of a hydroxyl group. When the haplophytine reduction product was treated with acetic anhydride in ether an unstable product was isolated which had a strong absorption band at 1735 cm.$^{-1}$ (acetoxy group). Acetylated haplophytine yielded the same product as haplophytine with sodium borohydride. The haplophytine reduction product was rather unstable and attempts to recrystallize it resulted in the extensive decomposition of the molecule. The cimicidine reduction product was recrystallized from ethanol and acetone, however, to give a highly solvated, white solid. The hygroscopic character of this product

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(2) E. I. du Pont de Nemours and Co., Inc., Fellow, 1950–1951.

(3) Procter and Gamble Company Fellow, 1952–1953.

(4) E. F. Rogers, H. R. Snyder and R. F. Fischer, THIS JOURNAL, **74**, 1987 (1952).

(5) H. R. Snyder, R. F. Fischer, J. F. Walker, H. E. Els and G. A. Nussberger, *ibid.*, **76**, 2819 (1954).

(6) H. L. Hergert and E. F. Kurth, *ibid.*, **75**, 1622 (1953).

interfered with analysis, but the results obtained indicated that the product probably resulted from the addition of two hydrogen atoms to cimicidine. Since the reduction of both alkaloids was similar and the attack took place in both cases at the carbonyl group absorbing at 1750 cm.$^{-1}$, it seems likely that the same, or a very similar, functional group was reduced in both alkaloids. Sodium borohydride is a reducing agent for aldehydes, ketones, acid chlorides, esters, lactones and some double bonds.[7] From the fact that apparently two hydrogen atoms were absorbed and the carbonyl group absorbing at 1750 cm.$^{-1}$ was involved, the reduction of a ketone group in both alkaloids was indicated. A ketone group with an absorption band at 1750 cm.$^{-1}$ would be expected to be part of a small-membered ring (the frequencies of absorption of the carbonyl of cyclobutanone, cyclopentanone and cyclohexanone are 1775, 1740 and 1715 cm.$^{-1}$, respectively,[8] and 17-ketosteroids show characteristic absorption around 1743 cm.$^{-1}$ [9]). No other chemical evidence for the presence of a ketone group is available. The ketone groups of the alkaloids, if present, were inert to such reagents as benzyl mercaptan, hydroxylamine, phenylhydrazine and malonic acid. The sodium borohydride reduction products of haplophytine and cimicidine could not be hydrogenated over platinum at room temperature and atmospheric pressure. The significance of this fact, since the alkaloids themselves are readily hydrogenated, and the hydrogenation products and the sodium borohydride reduction products are not identical, is not yet clear. Attempts to oxidize the sodium borohydride reduction product of haplophytine to haplophytine by several variations of the Oppenauer oxidation were unsuccessful. This result could possibly be due to instability of the alkaloid under the reaction conditions.

Experimental[10]

Cimicidine Hydrochloride.—This salt was prepared by a method similar to that employed in the preparation of haplophytine hydrochloride.[5] In contrast to the latter salt, cimicidine hydrochloride was very stable toward alcohols and water and could be crystallized from absolute ethanol–ether; m.p. 247–249° dec. (darkening from 225°).[11] Infrared absorption peaks were at 1710, 1677, 1598 and 1588 cm.$^{-1}$. For analysis, the sample was recrystallized twice from an absolute ethanol–ether mixture.

Anal. Calcd. for $C_{23}H_{28}O_5N_2$·HCl: C, 61.53; H, 6.51; N, 6.24. Found: C, 61.66; H, 6.69; N, 6.44.

Cimicidine, m.p. 257–260° dec., was isolated from its hydrochloride by dissolving in water, adjusting the *p*H to 8, extracting with chloroform, and crystallizing from ethanol.

Chloroplatinate.—When dilute chloroplatinic acid solution was added to a dilute hydrochloric acid solution of cimicidine, the solution turned golden yellow and, after eight to twelve hours, beautiful golden needles crystallized; m.p. 206–210° dec.[11] The chloroplatinate was soluble in alcohol and fairly soluble in water. It was recrystallized from dilute aqueous hydrochloric acid solution; the substance sintered at 205–208° and decomposed at 208–215°.[11]

Anal. Calcd. for $[C_{23}H_{28}O_5N_2$·HCl$]_2PtCl_4$: C, 44.73; H, 4.73; Pt, 15.8. Found: C, 44.42; H, 4.86; Pt, 15.8.

Attempts to recrystallize the chloroplatinate from water sometimes led to extensive decomposition, presumably due to hydrolysis. This difficulty was avoided when hydrochloric acid was present.

Picrate.—A saturated solution of picric acid in ether was added to a chloroform–ether solution of cimicidine until precipitation was complete. The bright-yellow powder was washed with ether and was crystallized from ethyl alcohol; m.p. 129–132°.[11] Two recrystallizations from commercial absolute alcohol gave beautiful clusters of yellow needles; m.p. 136–138°.[11] The picrate appeared to crystallize as a hydrate; it was dried *in vacuo* at 65° for five hours.

Anal. Calcd. for $C_{23}H_{28}O_5N_2$·$C_6H_3O_7N_3$·H_2O: C, 52.81; H, 5.04; N, 10.62. Found: C, 52.78, 53.35; H, 5.55, 5.37; N, 10.86.

The salt was then dried *in vacuo* for ten hours at 100°; further drying at 100° to constant weight caused a loss in weight of 1.9% (theoretical for 1 H_2O, 2.7%).

Anal. Calcd. for $C_{23}H_{28}O_5N_2$·$C_6H_3O_7N_3$: C, 54.29; H, 4.87. Found: C, 54.77; H, 4.70.

General and Qualitative Reactions.—Cimicidine did not react with carbon tetrachloride in the presence of chloroform even when allowed to stand for a week.[5] The sulfuric acid–phloroglucinol test for the methylenedioxy group, and the Duke tests for primary and secondary amines were also negative. Cimicidine decolorized 5% bromine in carbon tetrachloride and eventually gave an oily orange precipitate, which solidified to a yellow powder when covered with dry ether. The alkaloid did not, however, decolorize dilute alkaline permanganate solution in an hour; after this time, slight decolorization commenced.

When acetyl chloride was added to a benzene–ether solution, a white precipitate formed slowly. This precipitate was soluble in water, contained an ionic chlorine atom, and caused evolution of carbon dioxide from sodium carbonate. Unchanged cimicidine (confirmed by infrared absorption spectrum), m.p. 254–258° dec.[11] was recovered by chloroform extraction of the neutralized aqueous solution, followed by crystallization from ethanol.

No reaction took place between cimicidine and hydrazine hydrate in ethanol over a 0.5-hour reflux period. The unchanged alkaloid, m.p. 262–265° dec.,[11] was recovered in quantitative yield. The alkaloid was only slowly soluble in anhydrous hydrazine; an hour of vigorous reflux was necessary to dissolve 30 mg. of cimicidine in 4 ml. of the reagent. Solution took place with the formation of a yellow-brown color, and removal of the excess hydrazine *in vacuo* over sulfuric acid left only a brown resin, soluble in ethanol, but insoluble in ether or benzene.

A solution of 103 mg. of cimicidine in ether–benzene was treated with a large excess of ethereal diazomethane and allowed to stand overnight. The solution was evaporated, leaving a brown residue, which partly crystallized when treated with ethanol. The colorless crystals, m.p. 250–260° (darkening from 190°)[11] weighed 23 mg.; when recrystallized from ethanol the product decomposed at 258–261° and had an infrared absorption spectrum which proved it to be cimicidine.

Cimicidine also formed an amorphous aurichloride, which became semi-crystalline on standing; m.p. 173–175° dec.[11] It could not be recrystallized from dilute hydrochloric acid, and it decomposed somewhat when heated to 100° prior to analysis.

Anal. Calcd. for $C_{23}H_{28}O_5N_2$·HCl·$AuCl_3$: Au, 26.2. Found: Au, 26.9.

Attempted Reaction of Cimicidine with Cyanogen Bromide.—To a solution of 50 mg. of cyanogen bromide in 5 ml. of chloroform was added a solution of 150 mg. of cimicidine in 5 ml. of chloroform. The solution was let stand in the dark for 36 hours and was then extracted four times with 5-ml. portions of 2 *N* hydrochloric acid solution. The chloroform layer was taken to dryness on the steam-bath

(7) S. W. Chaikin and W. G. Brown, THIS JOURNAL, **71**, 122 (1949); M. L. Wolfrom and K. Anno, *ibid.*, **74**, 5583 (1952); M. Whaley and C. N. Robinson, *ibid.*, **75**, 2008 (1953).

(8) R. S. Rasmussen, "Progress in Chemistry of Natural Products," Vol. 5, Springer Verlag, Vienna, 1948, p. 331.

(9) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd edition, Reinhold Publ. Corp., New York, N. Y., 1949.

(10) Infrared spectra were determined and interpreted by Mrs. H. P. Leighly and Miss Helen Miklas using a Perkin–Elmer double beam spectrophotometer. Analyses were performed by Mr. Joseph Nemeth, Mrs. Lucy Chang, Mrs. Esther Fett, Mrs. Katherine Pih, Mrs. Emily Davis, Mrs. Rachel Kopel, Mrs. Jean Fortney and Clark Microanalytical Laboratories, Urbana, Ill.

(11) Melting point determined on a calibrated Fisher block.

under reduced pressure; the residue was a trace of a brown oil. The combined acid layers were neutralized with sodium carbonate and then extracted four times with 5-ml. portions of chloroform. The chloroform was removed from the combined chloroform layers on the steam-bath to leave a white residue. The residue was dissolved in hot ethanol and cooled; the characteristic white prisms of cimicidine slowly crystallized from the solution and were collected by vacuum filtration, m.p. 266–268° dec.[12] The recovery was 120 mg.

Reaction of Cimicidine with Methyl Iodide.—To a solution of 200 mg. of cimicidine in 20 ml. of dioxane were added 10 ml. of methyl iodide and 10 ml. of absolute ethanol. The solution was refluxed on the steam-bath for two hours and then heated at approximately 50° for 12 hours. The solvents were removed on the steam-bath under reduced pressure and the dark residue was crystallized from a mixture of ethanol and methyl iodide to give 110 mg. of dark red crystals. On a Kofler block the product melted at 155–157°, resolidified from 180–200° and then melted (dec.) at 270° (soft from 250°).

Anal. Calcd. for $C_{24}H_{33}N_2O_5I_3$: C, 35.66; H, 3.87; N, 3.47. Found: C, 37.12; H, 4.23; N, 3.56.

Hydrolysis of Cimicidine with Sulfurous Acid.—A saturated solution was prepared by passing sulfur dioxide through 10 ml. of water at 0°. To this acidic solution, in a Pyrex test-tube, was added 200 mg. of cimicidine and the tube was sealed. The cimicidine immediately dissolved to give a light yellow solution. The solution was heated on the steam-bath for three days, the tube was then opened, and the sulfurous acid and water removed on the steam-bath under reduced pressure to give a light yellow residue. A small portion of the residue was allowed to stand in the air and it slowly decomposed to an orange oil. The remainder of the yellow residue was dissolved in hot chloroform, the solution was filtered and concentrated to about 10 ml. and 1 ml. of ethanol added. Light yellow crystals slowly separated from the solution. When an attempt was made to collect the yellow crystalline material by filtration the crystals decomposed quickly to an orange oil on the filter. The remainder of the crystalline material was not collected by filtration; the chloroform was removed *in vacuo* and the light yellow residue was dissolved in water and extracted with chloroform at various *p*H values. Small amounts of colored material were extracted from the aqueous solution at *p*H 1, 7 and 10; the largest amount of solid material was in the extracts from the solution at *p*H 1. However, all the extract solution and the aqueous solution quickly turned deep orange in color and only orange oils could be isolated from any of them.

Hydrogenation of Cimicidine.—This hydrogenation was accomplished in methanol at 27° and 741 mm. over prereduced platinum oxide; the hydrogen absorbed in 12 minutes corresponded to 0.83 mole, and no further uptake was noted in an hour. At the completion of the hydrogenation, a voluminous precipitate of fine, silky needles completely filled the liquid. The precipitate was not very soluble in any organic solvents, but was finally separated from the platinum by dissolving in a large volume of methanol–acetone and filtering. The product was crystallized from methanol, again separating in large clumps of silky needles, m.p. 262–264° dec.[11] Infrared absorption maxima were at 1637, 1598 and 1588 cm.$^{-1}$.

The reduction of cimicidine was also conducted in 0.1 *N* sodium hydroxide solution at 27° and 745 mm. over prereduced platinum oxide; in this case 1.16 moles of hydrogen was taken up in 29 minutes and no further uptake was noted in an hour. The product was isolated by extraction of the neutralized (*p*H 8) solution with chloroform, followed by crystallization from methanol. The product separated in characteristic fine needles, m.p. 262–265° dec.,[11] and its infrared absorption spectrum was identical with that of the hydrogenation product obtained by neutral reduction; yield 58 mg.

Dihydrocimicidine.—This compound could be obtained crystalline only from methanol, from which it apparently crystallized as a dihydrate. The compound did not react with refluxing methyl iodide, nor did it appreciably decolorize dilute aqueous permanganate solution. For analysis, it was dried *in vacuo* at 100° for four hours.

(12) Melting point determined on a calibrated Hershberg type melting point apparatus using open capillaries; the initial temperature in each case was room temperature.

Anal. Calcd. for $C_{23}H_{30}O_5N_2 \cdot 2H_2O$: C, 61.32; H, 7.61; N, 6.22. Found: C, 61.56, 61.47; H, 7.24, 7.48; N, 6.58.

When an attempt was made to use ethanol as a crystallization solvent, the compound formed a yellow gel, which when heated became a white powder. The original dihydro product, m.p. 262–265° dec., was then obtained by crystallizing from methanol.

The picrate was formed by dissolving a small amount of the compound in absolute ethanol–ether and adding a slight excess of ethereal picric acid. The solution was warmed and concentrated, and when cooled, fine yellow needles, m.p. 160–180° dec., separated. The picrate was recrystallized twice from absolute alcohol, and the decomposition point became constant at 169–172°.[11]

Anal. Calcd. for $C_{23}H_{30}O_5N_2 \cdot C_6H_3O_7N_3$: C, 54.12; H, 5.17; N, 10.88. Found: C, 53.88; H, 5.46; N, 10.49.

No precipitate was formed when dry hydrogen chloride was passed into a solution of dihydrocimicidine in a chloroform–ether–benzene solvent or in ether–ethanol, possibly because of the solubilizing effect of the water of crystallization. The hydrochloride was obtained in crystalline form by allowing it to stand under ether–methanol for some days; brown granules slowly crystallized. Most of the color was removed by a quick washing with cold methanol, and the pale tan residual crystals were recrystallized from methanol–ether; m.p. 225–250° dec. One recrystallization from methanol–ether gave almost colorless clusters of very small needles, m.p. 245–260° dec.[11]

Anal. Calcd. for $C_{23}H_{30}O_5N_2 \cdot HCl$: C, 61.25; H, 6.93. Found: C, 61.86; H, 7.14.

Infrared absorption maxima were at 1724, 1630, 1598 and 1570 cm.$^{-1}$, there was also absorption characteristic of an amine hydrochloride. The product was water-soluble and gave an immediate precipitate with aqueous silver nitrate.

Since acid solutions of the compound appeared to be stable to water, an attempt was made to obtain the hydrochloride from aqueous solution. An analytically pure sample of dihydrocimicidine dihydrate was dissolved in dilute hydrochloric acid, and the resulting clear solution was evaporated to dryness *in vacuo* at room temperature. After two days, beautiful colorless prisms separated, m.p. 245–250° dec.[11] This product was water-soluble and contained an ionic chlorine atom. However, the analysis did not correspond to any simple derivative of dihydrocimicidine.

Anal. Found: C, 65.10; H, 7.13.

Alkaline Hydrogen Peroxide Reaction of Cimicidine.—Ninety-three milligrams of cimicidine was dissolved in 5 ml. of 1% sodium hydroxide solution, and 5 ml. of 30% hydrogen peroxide was added immediately. A white precipitate became evident within an hour, and the product was collected after 1.5, 3, 5, 8 and 18 hours. The combined precipitates weighed 51 mg.; they were soluble in chloroform and were crystallized from ethanol, separating as colorless rods, m.p. 294–297°[11] (little or no decomposition). For analysis, the product was recrystallized from absolute ethanol.

Anal. Calcd. for $C_{23}H_{28}O_6N_2$: C, 64.47; H, 6.74; N, 6.54. Found: C, 64.32; H, 6.23; N, 6.68, 6.71.

The cimicidine hydrogen peroxide product was still readily soluble in dilute aqueous alkali, and the solution turned dark on standing. The product was, however, insoluble in 2 *N* hydrochloric acid. It dissolved slowly and completely in the concentrated acid, but crystallized unchanged (as determined by infrared absorption spectrum) in fine rods, m.p. 293–297°,[11] when the strongly acid solution was poured into water.

Reaction of Haplophytine with Acetic Anhydride.—A solution of 280 mg. of haplophytine in 5 ml. of pyridine was treated with 30 drops of acetic anhydride and refluxed for 40 minutes. At the end of this time, part of the pyridine was removed by distillation, and the concentrated solution (2 ml.) was cooled. It was then treated with small portions of water and sodium bicarbonate (with cooling) until the evolution of gas ceased and the *p*H of the solution (*ca.* 10 ml.) was just 8. The product was extracted with four 5-ml. portions of chloroform, and the solvents were removed by heating *in vacuo*. Quite strong heating was necessary to remove the last of the pyridine. The yellow mass was then covered with ethanol, and crystallization usually commenced although in some cases a seed crystal of the pure compound

was necessary. The yield of crystalline product was 0.27 g., m.p. 187–190° (some softening from 175°),[11] 192–195°.[12] The material could be crystallized from ethyl acetate, but it could seldom be obtained completely colorless from this solvent. After two such recrystallizations, the specific rotation was constant at 27° (1–2%, chloroform). Considerable darkening was noted in chloroform, and it is recommended that this solvent not be used for the product in the future. It was also necessary to dry the product at low temperature, because it decomposed in an hour at 100° *in vacuo*. Absorption maxima in the infrared region were at 1750, 1710 and 1606 cm.$^{-1}$. A sample, m.p. 186–189°,[11] twice recrystallized from ethanol was dried at room temperature and then at 56° *in vacuo*.

Anal. Calcd. for $C_{27}H_{31}O_5N_3 \cdot CH_3CO_2H$: C, 64.80; H, 6.56; N, 7.82. Found: C, 64.71; H, 6.34; N, 7.82.

Another sample, m.p. 190–193°,[11] twice recrystallized from ethyl acetate was dried at 56° for four hours.

Anal. Found: C, 65.08; H, 6.16; N, 8.16.

The product was soluble in chloroform, benzene, acetone and water, and could be recovered unchanged from all these solvents, if removed quickly. It was somewhat soluble in ethyl acetate and alcohol, and slightly soluble in ether. It reacted almost immediately with carbon tetrachloride to give an insoluble product reminiscent of the corresponding haplophytine product.

When 113 mg. of the acetic anhydride product was treated with a large excess of diazomethane, no apparent decolorization of the reagent took place, and the original compound, m.p. 186–189°,[11] was recovered in 60% yield (twice-recrystallized basis). The infrared absorption spectrum was identical with that of the material before diazomethane treatment.

If the acetic anhydride product itself was dissolved in water and the *p*H adjusted to 10, the compound, m.p. 186–189°,[11] could be recovered in good yield by extraction with chloroform and crystallization from ethanol. If additional sodium carbonate was added to the solution, however, chloroform extraction and crystallization from alcohol gave haplophytine, m.p. 288–292° dec.[11] Attempts to remove the acetic acid (if present) with hydrochloric acid gave only brown acidic materials containing ionic chlorine. This chlorine could be removed with sodium bicarbonate to give a water-insoluble brown material which did not crystallize.

To determine whether the anhydride was necessary for the reaction, haplophytine was dissolved in aqueous acetic acid and warmed in the presence of pyridine. The *p*H of the solution was adjusted to 8, and it was extracted three times with chloroform. The extracts were crystallized from ethanol and proved to be unchanged haplophytine, m.p. 283–287° dec. In another attempt, haplophytine was refluxed with pyridine and propionic acid, duplicating the conditions of the anhydride reaction, but only unchanged haplophytine was recovered (in good yield).

Reaction of Haplophytine with Butyric Anhydride.—Two hundred milligrams of haplophytine was dissolved in 5 ml. of dry pyridine and treated with 20 drops of freshly distilled butyric anhydride (b.p. 191–193°). The reaction mixture was refluxed for 20 minutes, during which time it darkened appreciably. Most of the pyridine was then removed by distillation, the solution was cooled, and water and sodium carbonate were added carefully to *p*H 8. The solution was extracted three times with 5-ml. portions of chloroform, and the solvents were removed by distillation, strong heating being necessary to remove the last traces of pyridine. The yellow-brown product was dissolved in ether and filtered to remove a small amount of black material. Evaporation of the ether gave a yellow resin, which was taken up in water, leaving a small amount of a yellow oil. The yellow aqueous solution was evaporated almost to dryness *in vacuo* at room temperature, and a few drops of alcohol were added. Then 5 ml. of ether was added, and the system separated into two phases. When this system was warmed gently, crystallization began and was allowed to continue overnight.

The product, m.p. 165–167°, was separated by filtration and weighed 0.146 g. Its infrared absorption spectrum was very similar to that of the acetic anhydride product. Two recrystallizations from ether gave colorless prisms, m.p. 167–169°.[11] The product was dried for five hours at 56° *in vacuo*.

Anal. Calcd. for $C_{31}H_{38}O_6N_3$: C, 67.86; H, 6.98; N, 7.66. Found: C, 67.43; H, 7.05; N, 7.43.

In an attempt to prepare a hydrochloride of this compound, 77 mg. of the product was dissolved in ether–benzene and treated with dry hydrogen chloride. The precipitate was centrifuged and washed twice with ether. The washings were evaporated to dryness, and no odor of butyric acid was noted. The hydrochloride was dried *in vacuo* and it weighed 96 mg. It turned pink, however, on standing, and rapidly resinified. Nothing crystalline could be obtained when it was treated (after some decomposition had taken place) with dry ammonia gas.

Reaction of Haplophytine with Benzoic Anhydride.—A mixture of 227 mg. of haplophytine and 130 mg. of benzoic anhydride, m.p. 41–42°, was dissolved in 5 ml. of pyridine and refluxed for one-half hour. Most of the pyridine was removed by distillation, and the remaining solution was cooled and treated with water and sodium bicarbonate to *p*H 8 (volume, 10 ml.). It was then extracted three times with 5-ml. portions of chloroform, and the solvents were removed by heating *in vacuo*. The yellow mass obtained was difficult to crystallize; it was quite soluble in ether and could be obtained as a white powder, m.p. 190–195°,[13] from this solvent; yield 138 mg. In one case, colorless prisms were obtained, m.p. 190–195°, but these dissolved in a little ether and could only be recovered as the powder, m.p. 190–195°. This material was recrystallizable from petroleum ether (b.p. 90–105°) and was again obtained as a white powder, m.p. 188–191°,[11] $[\alpha]^{25}_D$ +48° (1%, chloroform).

Anal. Found: C, 74.24; H, 6.95; N, 7.81; CH_3O-, 5.86.

The infrared absorption spectrum had bands at 1747, 1656 and 1605 cm.$^{-1}$, and the compound reacted very quickly with carbon tetrachloride. It was very soluble in ethanol but when allowed to stand in this solvent for some time, colorless needles, m.p. 260–263° dec., were deposited. These needles could be recrystallized from ethanol and after two such recrystallizations, decomposed constantly at 255–260° (darkening from 220°). The reaction was repeated, and there was obtained 199 mg. of the product, m.p. 240–245° dec.,[11] from 220 mg. of haplophytine and 130 mg. of benzoic anhydride when the crude reaction product was taken up immediately in ethanol. One recrystallization gave 101 mg. of fine needles decomposing at 255–258°.[11] Infrared absorption peaks were at 1745, 1718–1700 and 1604 cm.$^{-1}$. A total of 50 mg. of unchanged haplophytine was recovered from the mother liquors. For analysis, the benzoic anhydride product was recrystallized twice from absolute ethanol.

Anal. Calcd. for $C_{34}H_{35}O_6N_3$: C, 70.20; H, 6.07; N, 7.22. Found: C, 70.01; H, 6.34; N, 7.00.

This product was completely insoluble in 0.1 *N* sodium hydroxide and could be separated from haplophytine on this basis. It dissolved slowly in 3% alkali (three days) with formation of a yellow solution. In this case, extraction of the neutralized solution with chloroform and crystallization of the extracted material from ethanol afforded haplophytine, m.p. 283–287° dec.[11]; this was confirmed by infrared analysis. The benzoic anhydride product was also less soluble in acids than was haplophytine, but it dissolved eventually. When acetic or hydrochloric acid solutions were evaporated to dryness, neutralized and taken up in ethanol, small amounts of crystalline materials were obtained, but it has not yet been possible to separate them from the accompanying resins.

Reaction of Dihydrocimicidine with Acetic Anhydride.—Sixty milligrams of the compound was dissolved in 4 ml. of pyridine and treated with eight drops of acetic anhydride. The reaction mixture was refluxed for 30 minutes and worked up as with the cimicidine–acetic anhydride product. The reaction mixture, in this case, crystallized as the last of the pyridine was removed; m.p. 230–240° dec. The product, which weighed 46 mg., was slowly but completely soluble in water. The aqueous solution was filtered to remove a small amount of extraneous material and evaporated to dryness *in vacuo* at room temperature. The colorless product was crystallized from methanol and separated as very fine needles, m.p. 225–235° dec.[11] Absorption maxima in the infrared were at 1785, 1690 and 1616 cm.$^{-1}$. The analytical sample was recrystallized from methanol and dried for 20 hours at 56° *in vacuo*.

Anal. Calcd. for $C_{25}H_{32}O_6N_2$: C, 65.77; H, 7.07; N, 6.14. Found: C, 65.66; H, 6.64; N, 6.21.

About 25 mg. of the product was dissolved in 4 ml. of 0.1

N hydrochloric acid and evaporated to dryness. The resulting material was very soluble in methanol or acetone, but could be crystallized from methanol–ether or, better, from methanol–ethyl acetate. Large crystals, totaling 19 mg., were obtained.

Anal. Calcd. for $C_{25}H_{32}O_6N_2 \cdot HCl \cdot H_2O$: C, 58.76; H, 6.90; N, 5.48. Found: C, 57.83; H, 6.82; N, 5.44.

The material was water-soluble and contained ionic chlorine as determined by the immediate formation of a precipitate with silver nitrate.

Reaction of Cimicidine with Acetic Anhydride.—Eighteen drops of acetic anhydride was added to 176 mg. of cimicidine dissolved in 5 ml. of pyridine. The reaction mixture was refluxed for 35 minutes, after which most of the pyridine was removed by distillation. The concentrate was cooled, and water and sodium carbonate were added carefully to *p*H 8. The solution (10 ml.) was extracted three times with chloroform, this solvent was removed, and the yellow oil was taken up in ether. The product crystallized very slowly the first time, but in later runs it was obtained readily by seeding the ether solution. The yield was 164 mg. of colorless needles, m.p. 172–173°, $[\alpha]^{25}D$ −105° (1.5%, methanol). Absorption maxima in the infrared region were at 1774, 1751, 1671 and 1602 cm.$^{-1}$. The material was soluble in benzene, chloroform, ethanol, ethyl acetate, acetone and water. Evaporation of the water solution gave a resin, which when covered with ether and seeded, crystallized in fine needles, m.p. 170–171°. The compound would crystallize only from ether, and a sample was recrystallized twice from this solvent for analysis; m.p. 173–174°.[11]

Anal. Calcd. for $C_{25}H_{30}O_6N_2$: C, 66.06; H, 6.65; N, 6.17. Found: C, 66.78; H, 6.80; N, 6.08.

A small amount of the compound was dissolved in water and treated with 0.1 *N* sodium hydroxide. The solution was neutralized, extracted with chloroform, and the product was crystallized from ethanol. It separated in prisms, m.p. 258–261° dec.,[11] and its infrared absorption spectrum was identical with that of cimicidine.

Ninety-six milligrams of the acetic anhydride product was dissolved in 5 ml. of 0.1 *N* hydrochloric acid and evaporated to dryness at room temperature *in vacuo*. The product was taken up in 2 ml. of methanol, and 5 ml. of acetone was added. A powdery material separated which crystallized in granules when seeded with a granule obtained in a previous small-scale run; m.p. 240–250° dec. Two successive recrystallizations from methanol–acetone raised the decomposition point to 260–265° (darkening from 240°).[11] Infrared absorption maxima were at 1770, 1721, 1683 and 1608.

Anal. Calcd. for $C_{25}H_{30}O_6N_2 \cdot HCl$: C, 61.15; H, 6.37; N, 5.71. Found: C, 61.26; H, 6.70; N, 5.48.

Twenty milligrams of the hydrochloride was dissolved in water and treated with sodium bicarbonate (gas evolution) to *p*H 7. The solution was extracted with chloroform, the solvent was removed, and the product was taken up in ether. It slowly crystallized and melted at 167–172°. One recrystallization from ether gave needles, m.p. 174–176°, which on admixture with the original cimicidine–acetic anhydride product melted at 173–176°. Neither this product nor its hydrochloride gave a precipitate with chloroplatinic acid, and on long standing the solution darkened appreciably.

Reaction of Cimicidine with Butyric Anhydride.—Fifteen drops of freshly distilled butyric anhydride was added to 136 mg. of cimicidine in 5 ml. of pyridine, and the reaction mixture was refluxed for 20 minutes. It was worked up as was the acetic anhydride reaction mixture, but no crystalline material could be obtained, except 30 mg. of unchanged cimicidine. The bulk of the product was a colorless material soluble in water, alcohols, benzene and ether. It was insoluble in petroleum ether but remained a colorless oil.

Treatment of Haplophytine with Diazomethane.—A solution of 0.18 g. of haplophytine in ether–chloroform was treated for 16 hours with a total of 0.5 g. of diazomethane in ether, prepared by the method of Arndt.[13] No apparent decolorization of the reagent was noted, and 0.16 g. of haplophytine was recovered. Two recrystallizations from 95% ethanol gave typical clusters of needles, m.p. 290–293° dec.[11] The infrared absorption spectrum was identical with that of authentic haplophytine.

Anal. Calcd. for $C_{27}H_{31}O_5N_3$: C, 67.91; H, 6.54. Found: C, 67.90; H, 6.26.

A similar experiment was conducted in the presence of methanol, in accordance with the directions of Schoenberg[14]; in this case, 0.14 g. of the alkaloid was used. Evaporation of the reaction mixture gave a brown resinous product, which, when taken up in ethanol, slowly deposited 40 mg. of granular crystals, m.p. 250–255° dec., in three crops. One recrystallization from ethanol gave clusters of needles, m.p. 278–281° dec.,[11] which reacted almost immediately with carbon tetrachloride in the presence of chloroform. The infrared absorption spectrum proved that this material was unchanged haplophytine.

Reduction of Haplophytine with Sodium Borohydride.—To a solution of 270 mg. of haplophytine in 10 ml. of dioxane–ethanol was added a solution of 150 mg. of sodium borohydride in 5 ml. of ethanol. The solution was let stand overnight and was then neutralized with 2 *N* hydrochloric acid solution. The solvents were removed on the steam-bath under reduced pressure and the residue was triturated with absolute ethanol and filtered. The ethanol was removed on the steam-bath under reduced pressure and the residue was again triturated with ethanol and the ethanol removed as before to leave a white hygroscopic powder. All attempts at recrystallization of the reduction product caused extensive decomposition. The infrared spectrum of the reduction product had no absorption band at 1750 cm.$^{-1}$ (present in haplophytine) and there was a broad absorption band at 3360 cm.$^{-1}$.

A few milligrams of the reduction product was suspended in ether and a few drops of acetic anhydride were added. The reduction product slowly dissolved. Removal of the ether under reduced pressure yielded a small amount of a white, rather unstable, solid. The infrared spectrum of this product had a strong absorption band at 1735 cm.$^{-1}$. All attempts at purification of this product resulted in extensive decomposition.

Acetylated haplophytine was reduced with sodium borohydride under identical conditions. The product had an identical infrared spectrum to that of the haplophytine reduction product. The instability of the reduction product again prevented purification.

Reduction of Cimicidine with Sodium Borohydride.—To a solution of 120 mg. of cimicidine in 25 ml. of absolute ethanol was added a solution of 25 mg. of sodium borohydride in 10 ml. of absolute ethanol. The solution was let stand at room temperature for 3.5 hours and then the alcohol was removed on the steam-bath under reduced pressure. The white residue was dissolved in water and the *p*H was adjusted to 1 with 2 *N* hydrochloric acid solution and then to 7–8 with 5% sodium hydroxide solution. The aqueous solution was extracted six times with 5-ml. portions of chloroform. The combined chloroform layers were diluted to 100 ml. with chloroform and dried over sodium sulfate. The chloroform was then removed on the steam-bath under reduced pressure to yield 90 mg. of a white residue. The material was recrystallized from ethanol; the material came out of ethanol as a highly solvated, almost gelatinous mass which on filtration formed a white solid, m.p. 274–276° dec. (dark from 255°).[12] The infrared spectrum was significant in that the 1750 cm.$^{-1}$ absorption band of cimicidine had disappeared and that absorption due to a hydroxyl group was apparent. The reduction product was also recrystallized from acetone; it separated as a bulky, cottony mass which lost volume on filtration, m.p. 270–273° dec. (dark from 260°).[12]

The reduction product was dried at 100° (1 mm.) prior to analysis but it was extremely hygroscopic and gained weight while being weighed out for analysis; it appeared to come to constant weight and the analytical result was calculated for a monohydrate. The reduction product was subsequently analyzed as a hygroscopic material and this analytical result was calculated for an anhydrous material. In neither case was a good analysis obtained, although the analysis was closer to the calculated formulas than to any other conceivable formula.

Anal. Calcd. for $C_{23}H_{30}O_5N_2 \cdot H_2O$: C, 63.87; H, 7.46. Found: C, 64.36; H, 7.39. Calcd. for $C_{23}H_{30}O_5N_2$: C, 66.67; H, 7.30. Found: C, 67.23; H, 7.52.

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