Anal. Calcd. for $C_{17}H_{17}Cl_2NO$: C, 63.36; H, 5.32. Found: C, 62.94; H, 5.32.

Pyrolysis of 1,1-Bis-(*p*-chlorophenyl)-2-propanoneoxime Tosylate.—Oxime tosylate (2.8 g., m.p. 86–87°, 1 deg. per min.) was heated in a short-path still equipped with a Dry Ice cooled receiver. The temperature of the heating-bath was 100° at the start and was allowed to rise over a 20-minute period to 160°. A colorless distillate (0.20 g.) which was miscible with water was obtained. This was demonstrated to be acetonitrile from its boiling and melting points and through its conversion to acetophenone. The latter compound was converted to a 2,4-dinitrophenylhydrazone which was compared to an authentic sample, m.p. 240–241° (dec.), m.m.p., 240–241° (dec.).

Preparation and Rearrangement of 1,1-Diphenylacetoneoxime Tosylate.—The oxime of 1,1-diphenylacetone (4.5 g., m.p. 164–165°)[16] was converted to the tosylate ester by a procedure analogous to that employed for the preparation of *p,p'*-dichlorodesoxybenzoinoxime tosylate from the corresponding oxime; yield 72%, m.p. 72–73° (dec., bath preheated to 70°).

Anal. Calcd. for $C_{22}H_{21}NSO_3$: C, 69.62; H, 5.58. Found: C, 69.74; H, 5.60.

This oxime ester (0.95 g.) in 10 ml. of pyridine was heated at reflux for 15 minutes. The mixture was then poured into 300 ml. of water. The precipitate that separated was recrystallized from benzene to give 0.30 g. of white needles, m.p. 133–136°. Two more recrystallizations of the substance gave m.p. 147°, which corresponds to the melting point of N-acetylbenzhydrylamine.[17] This compound when warmed with concentrated hydrochloric acid and a little ethanol gave a pronounced odor of ethyl acetate.

(16) R. Stoermer, *Ber.*, **39**, 2302 (1906).

(17) M. Busch and L. Leefhelm, *J. prakt. Chem.*, **72**, 14 (1908).

LOS ANGELES, CALIFORNIA

[CONTRIBUTION FROM THE BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, AGRICULTURAL RESEARCH ADMINISTRATION, UNITED STATES DEPARTMENT OF AGRICULTURE]

Alkaloids from *Tripterygium wilfordii* Hook. The Structure of Wilforine, Wilfordine, Wilforgine and Wilfortrine

BY MORTON BEROZA

RECEIVED MAY 1, 1952

All the fragments resulting from the degradation of the ester alkaloids wilforine, wilfordine, wilforgine and wilfortrine have been isolated, and the formula for the sum of the components of each alkaloid is in agreement with the molecular formula calculated from the elementary analyses of each alkaloid. It has been shown by elementary analysis, paper chromatography and X-ray diffraction patterns that the four alkaloids possess the same polyhydroxy nucleus, $C_{15}H_{26}O_{10}$. This nucleus contains ten hydroxyl groups, but only eight of them are esterified in the intact alkaloid—five with acetic acid, one with either benzoic or 3-furoic acid, and two with a nitrogen-containing dicarboxylic acid. Wilforine and wilforgine possess the same dicarboxylic acid and wilfordine and wilfortrine possess the same dicarboxylic acid, but the latter acid is the hydroxy congener of the former. Permanganate oxidation of both dicarboxylic acids gives acetic, oxalic and quinolinic acids. The dicarboxylic acids are 2-substituted nicotinic acid derivatives, and the probable formulas of these acids are given.

Wilfordine, the insecticidal alkaloid isolated from the roots of *Tripterygium wilfordii* Hook,[1] has been shown by countercurrent distribution to be a mixture.[2] From this mixture four ester alkaloids, designated wilforine, wilfordine, wilforgine and wilfortrine[3,4] have been isolated by partition chromatography employing ultraviolet absorbancy ratios.[2] Countercurrent distribution patterns of the alkaloids indicate that they were pure. Upon saponification wilforine and wilfordine yielded 1 mole of benzoic acid, 5 moles of acetic acid and 2 moles of steam non-volatile acid per mole of alkaloid, whereas wilforgine and wilfortrine yielded 1 mole of 3-furoic acid, 5 moles of acetic acid and 2 moles of steam non-volatile acid per mole of alkaloid.

In addition to acetic and benzoic acids, the saponification of wilforine yielded a steam non-volatile nitrogeneous dibasic acid of molecular formula $C_{11}H_{13}O_4N$, m.p. 195–196°. From wilforgine an identical acid was obtained.

A steam non-volatile nitrogeneous dibasic acid was also obtained from wilfordine and wilfortrine. These acids, which were found to be identical, had a molecular formula of $C_{11}H_{13}O_5N$, m.p. 178–179°.

The solutions remaining after the removal of the acids were neutralized and evaporated to dryness, and the residue was extracted with hot methanol. Evaporation of these extracts yielded in each case the same polyhydroxy nucleus, whose molecular formula is $C_{15}H_{26}O_{10}$. The compound does not have a definite melting point, but darkens as the temperature is slowly raised above 240°. It shows no absorption in the ultraviolet.

The sums of the component parts of wilforine, wilfordine, wilforgine and wilfortrine are in agreement with the respective formulas calculated from the elementary analyses on the intact alkaloids.

Experimental[5]

The operations in the structure studies are summarized in the diagram.

Isolation of Acids from Saponified Alkaloid.—The alkaloids were saponified as already described.[3,4] The neutralized saponification mixture was made alkaline to phenolphthalein with a few drops of 0.1 *N* sodium hydroxide and then continuously extracted with ether in an all-glass apparatus for 24 hours to remove the diethylene glycol. The ether extract was discarded. The extracted water solution was carefully acidified with dilute sulfuric acid to *p*H 2.8 and continuously extracted with ether for at least 24 hours. A soda lime tube was used to exclude atmospheric carbon dioxide. If the *p*H of the water solution after the ether extraction differed much from 2.8, it was readjusted to this *p*H and again extracted for 24 hours. Extraction was complete when the ultraviolet absorbancy of the water solution was low: ml. × absorbancy at 272 mμ ÷ 26 = mg. dibasic acid (approximate). The water solution contained the polyhydroxy nucleus of the alkaloid and was held aside to be worked up as described below. The ether extract, which

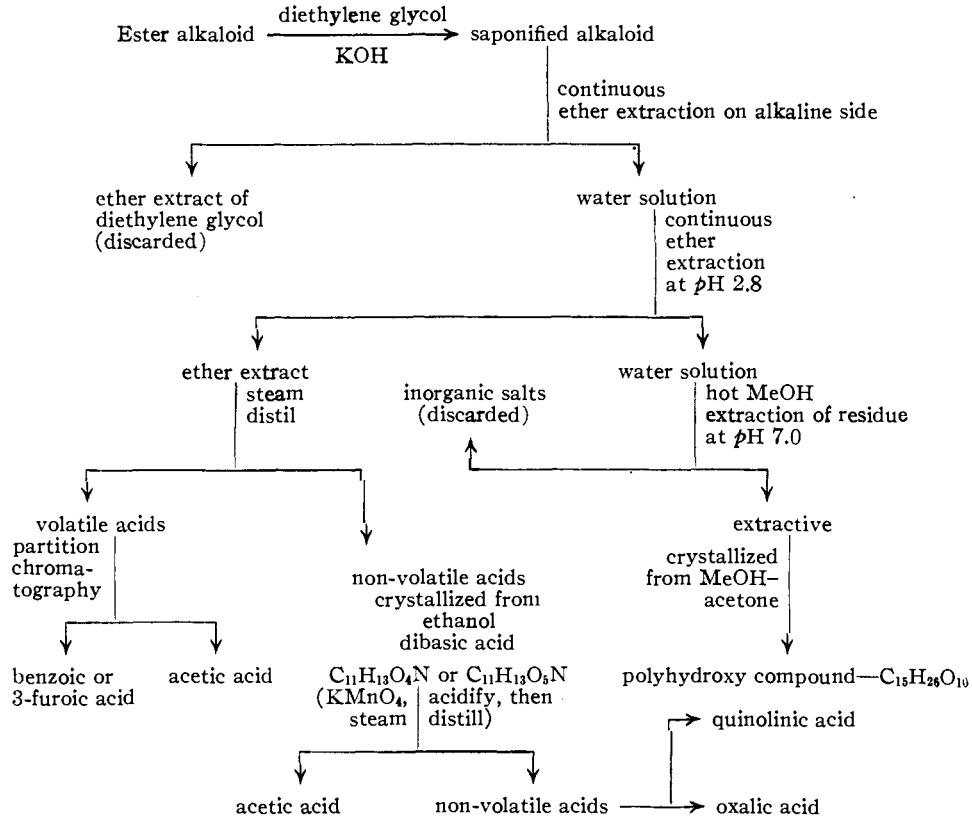
(1) F. Acree and H. L. Haller, THIS JOURNAL, **72**, 1608 (1950).

(2) M. Beroza, *Anal. Chem.*, **22**, 1507 (1950).

(3) M. Beroza, THIS JOURNAL, **73**, 3656 (1951).

(4) M. Beroza, *ibid.*, **74**, 1585 (1952).

(5) All m.p.'s are corrected. Determinations of carbon, hydrogen and nitrogen were made by Oakwold Laboratory, Alexandria, Virginia.



contained all the acids resulting from saponification of the alkaloid, was titrated to determine the total amount of acid. This result was usually a little low, probably owing to some entrainment loss when the solution was extracted with ether on the alkaline side. In the procedure previously used[3,4] the neutralized saponification solution was acidified and steam distilled without prior removal of the diethylene glycol. This procedure, while giving more accurate results, was unsatisfactory because it subjected the polyhydroxy compound to prolonged heating with steam in acid solution.

The neutralized total acids were transferred to an all-glass steam distillation apparatus, acidified to congo red with sulfuric acid, and steam distilled exhaustively. The distillate contained the volatile acids, which could be separated and determined chromatographically as already described.[3,4]

The solution of the steam-non-volatile residue, which contained the dibasic acid of the alkaloid, was adjusted to *p*H 2.8 and extracted with ether continuously for at least 24 hours. The ether extractive was dissolved in a minimum of hot 95% ethanol, and the resulting solution was filtered, concentrated to a small volume, and put in an ice-box to crystallize. The dibasic acid of the alkaloid crystallized very slowly, and was recrystallized from 95% ethanol to a constant melting point.

Isolation of the Polyhydroxy Nucleus.—The water solution containing the polyhydroxy nucleus was adjusted carefully with dilute sodium hydroxide to *p*H 7.0 and evaporated to dryness on a 40° water-bath under reduced pressure. The residue was triturated and extracted repeatedly (about five times) with hot absolute methanol. The filtered methanol extract was evaporated to dryness, weighed, redissolved in hot methanol, filtered, concentrated to a few milliliters and finally an equal volume of acetone was added. The compound crystallized very slowly; after two more crystallizations a pure colorless compound was obtained.

The Dibasic Acids.—In two preliminary determinations on 32.0 mg. and 29.5 mg. of wilforine, the continuous ether extract of the acidified steam non-volatile residue contained (after correcting for a blank run) 1.90 and 1.78 equivalents of acid per mole of alkaloid, respectively. In a large degradation run on 1.05 g. of wilforine, there was isolated from this fraction 0.1155 g. of a nitrogen-containing acid that melted at 194.5–195.5° and 0.0254 g. of less pure material that melted several degrees lower; total yield 52%. The purest material obtained from another run melted at 195.5–196°. A potentiometric titration of the acid with alkali indicated that the acid was dibasic. The dibasic acids were dried at 80° under high vacuum (oil-pump) for analysis.

Anal. Calcd. for $C_{11}H_{13}O_4N$: C, 59.10; H, 5.83; N, 6.28; mol. wt., 223.2; neut. equiv. (dibasic acid), 111.6. Found: C, 59.34, 59.58; H, 5.78, 5.83; N, 6.06; mol. wt. (from N), 231; neut. equiv., 116.7.

From 30.8 mg. of wilfordine 1.79 equivalents of acid per mole of alkaloid were obtained. In a run on 0.8090 g. of wilfordine 92.4 mg. of a nitrogen-containing acid (42% of theoretical) that melted at 178–179° was isolated. The potentiometric titration of this acid likewise was typical of a dibasic acid.

Anal. Calcd. for $C_{11}H_{13}O_5N$: C, 55.2; H, 5.48; N, 5.86; mol. wt., 239.2; neut. equiv. (dibasic acid), 119.6. Found: C, 55.18; H, 5.62; N, 5.84; mol. wt. (from N), 240; neut. equiv., 121.5.

From a 30.57-mg. sample of wilforgine 1.90 equivalents of acid per mole of alkaloid was obtained. From a 0.2984-g. sample of wilforgine 20.7 mg. of acid melting at 193.5–195° and 11.9 mg. of acid melting several degrees lower were isolated; total yield 42%. The melting point was not depressed when this acid was mixed with the dibasic acid of wilforine.

Anal. Calcd. for $C_{11}H_{13}O_4N$: C, 59.10; H, 5.83; N, 6.28; neut. equiv. (dibasic acid), 111.6. Found: C, 59.10; H, 5.90; N, 6.94; neut. equiv., 116.5.

From a 32.5-mg. lot of wilfortrine 2.16 equivalents of acid per mole of alkaloid was obtained. From 0.1545 g. of wilfortrine 1.83 equivalents of acid per mole of alkaloid was isolated, and from this fraction 10 mg. of dibasic acid (24% of theory) melting at 176.5–177.5° was obtained. A micro carbon–hydrogen analysis on an undersized sample (2.159 mg.) was run, but no nitrogen analysis was made owing to lack of material. The melting point of this acid was not depressed when mixed with the dibasic acid of wilfordine.

Anal. Calcd. for $C_{11}H_{13}O_5N$: C, 55.2; H, 5.48; neut. equiv. (dibasic acid), 119.6. Found: C, 56.37; H, 5.39; neut. equiv., 124.

Paper Chromatography of the Dibasic Acids.—Strips of Whatman No. 1 paper 5 mm. by 400 mm. were washed in

order with 1% oxalic acid, water and ethanol to eliminate ghost spots[6] and then allowed to dry at room temperature in a hood overnight. At 8 cm. from one end about 50 μg. of the acid was placed in a 0.5-cm. diameter area. The strips, with no conditioning, were developed by the ascending technique of Williams and Kirby.[7] The following solvents, prepared fresh for each run, have been used for the chromatography of acids[8]: (A) chloroform, 1 ml.; 95% ethanol, 1 ml.; 90% formic acid, 2%. (B) Phenol, 3 g.; water, 1 ml.; 90% formic acid, 1%. (C) Isoöctane, 4 ml.; 95% ethanol, 4 ml.; acetone, 1 ml.; 90% formic acid, 1%.

After development the solvent front was marked. With solvents A and C the solvent fronts were not easily visible but could be seen in the dark under ultraviolet light when the strips were dry. The strips were dried overnight in a hood, and the position of the acids was revealed by spraying with a 0.04% alcoholic solution of brom phenol blue. To establish the identity of compounds, the strips containing the compounds were run at the same time. The results ($R_F \times 100$) of chromatographing the dibasic acids from each alkaloid with the three solvents are summarized below:

	A	B	C
Wilforine	92	89	93
Wilforgine	91	88	92
Wilfordine	72	85.5	76
Wilfortrine	69	85.5	76

There is no significant difference in R_F values between the dibasic acids of wilforine and wilforgine or between the wilfordine and wilfortrine dibasic acids.

X-Ray Diffraction Patterns of the Dibasic Acids.—The patterns[9] were determined by the powder method with a cylindrical camera. The Straumanis technique[10] was used. The crystals were held on a glass fiber with library paste. The patterns were produced on 35-mm. photographic film in cameras of 114.6 mm. effective diameter by nickel-filtered copper radiation, $K\alpha$ wave length 1.539 Å. The d/n (interplanar spacing/order of diffraction) values were calculated from the principal lines.

A summary of the X-ray diffraction data on the dibasic acids is given in Table I. There is no significant difference between the patterns of the dibasic acids of wilforine and wilforgine or between the patterns of the wilfordine and wilfortrine dibasic acids.

TABLE I

d/n VALUES[a] AND INTENSITY OF LINES[b] FROM X-RAY DIFFRACTION PATTERNS OF THE DIBASIC ACIDS FROM THE VARIOUS ALKALOIDS

Wilforine	Wilforgine	Wilfordine	Wilfortrine
10.26 s	10.20 s	10.76 m	10.63 m
8.92 vw	8.88 vw	9.39 vw	9.39 vw
7.13 vw	7.24 vw	7.07 m	7.01 m
5.09 s	5.09 s	5.53 w	5.50 w
4.47 w	4.50 w	5.33 w	5.33 w
4.16 vw	4.18 vw	4.61 s	4.61 s
3.76 s	3.79 s	4.16 vw	4.14 vw
3.60 vw	3.60 vw	3.72 s	3.72 s
3.43 vw	3.45 vw	3.33 w	3.33 w
3.20 vw	3.21 vw	2.79 vw	2.78 vw
2.61 vw	2.63 vw	2.57 vw	2.58 vw
2.53 w	2.54 w	2.47 vw	2.46 vw
2.44 w	2.45 w	2.375 vw	2.375 vw
2.35 vw	2.35 vw	2.13 vw	2.12 vw

[a] d = interplanar spacing in ångström units; n = order of diffraction. [b] s = strong, m = medium, w = weak, v = very.

(6) E. P. Kennedy and H. A. Barker, *Anal. Chem.*, **23**, 1033 (1951).

(7) R. J. Williams and H. Kirby, *Science*, **107**, 481 (1948).

(8) J. B. Stark, A. E. Goodban and H. S. Owens, *Anal. Chem.*, **23**, 413 (1951).

(9) The patterns were made by E. L. Gooden of this Bureau.

(10) M. Straumanis and A. Ievins, *Naturwissenschaften*, **23**, 833 (1935).

Ultraviolet Absorption Spectra of the Dibasic Acids.—The ultraviolet absorption curves were made with a Beckman model DU quartz spectrophotometer. The spectra of the four dibasic acids and those of their sodium salts (Figs. 1 and 2) are remarkably similar, a result which demonstrates the close similarity of these compounds.

Other Tests on the Dibasic Acids.—The addition of a ferrous sulfate solution to a small amount of each of the dibasic acids produced no appreciable color. This result indicates the absence of a carboxyl group ortho to the pyridine nitrogen.[11]

The addition of a ferric chloride solution to a solution containing the dibasic acid of wilforine produced a faint yellow solution. A much yellower solution was produced under the same conditions by the dibasic acid of wilfordine. The latter yellow color, which was as intense as that produced by tartaric acid under the same conditions, indicates the presence of an α-hydroxy acid.[12]

Oxidation of the Dibasic Acids of Wilforine and Identification of the Products.—A solution of 87.1 mg. of dibasic acid of wilforine in 25 ml. of water was made slightly alkaline to phenolphthalein and 0.8 g. of finely powdered potassium permanganate (large excess) was added. After refluxing overnight, the excess permanganate was destroyed by passing sulfur dioxide into the solution. The manganese dioxide was filtered off and washed with hot water. The combined filtrate and washings were brought to a boil to dispel excess sulfur dioxide, then made alkaline and concentrated to about 15 ml. The solution was then acidified to pH 2.0 with sulfuric acid and continuously extracted with ether until acid no longer was extracted. The extract was then exhaustively steam distilled.

The distillate was made alkaline and evaporated to dryness. The acidified residue was then chromatographed[13] and only one zone containing an appreciable amount of acid (0.89 equiv. per mole of dibasic acid) was obtained. This acid had the same threshold volume as acetic acid. It was made up to 50 ml. and steam distilled at constant volume according to the procedure described for determining distillation constants and curves.[14] Fractions containing 17 ml. were collected. The results, which gave a straight line plot on semi-log paper, were in good agreement with those obtained for acetic acid. Known acetic acid: 17 ml., 18.5% of acid; 34 ml., 33.2; 51 ml., 45.6; 68 ml., 55.2. Acid from the chromatography: 17 ml., 18.2; 34 ml., 32.8; 51 ml., 44.5; 68 ml., 54.0.

The steam non-volatile acidic residue was adjusted to pH 2.0 and extracted continuously with ether for at least 24 hours. The extract contained 3.15 equivalents of acid per mole of dibasic acid. The neutralized extract was again acidified and continuously extracted with ether. After the ether was evaporated off, a water solution of the acids remained. This solution was transferred to a test-tube and the water evaporated in a current of dry air while the test-tube was immersed in a steam-bath. Unfortunately some of the acid was lost, as an acid sublimate deposited on the air-inlet tube. An aliquot of the residue was therefore sublimed at reduced pressure (water-pump) in a microsublimator. The temperature was gradually raised and then held at 100–110° for about half an hour. The sublimate that formed was washed off with acetone, and when the solution was slowly concentrated crystals separated. After drying the crystals melted unsharply about 180° (sealed capillary) and decolorized a dilute solution of potassium permanganate. A mixed melting point with a sample of oxalic acid that melted at 186° was not depressed (181–183.5°). The sublimate was therefore impure oxalic acid.

The temperature of the residue in the microsublimator was gradually raised to and held at 160° for about an hour. More material sublimed, but this material could not be characterized.

Since oxalic acid was present, a quantitative determination of this acid was made. An aliquot of the residue in a small centrifuge tube was made ammoniacal and calcium

(11) R. C. Elderfield, "Heterocyclic Compounds," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1950, p. 569.

(12) F. Schneider, "Qualitative Organic Microanalysis," John Wiley and Sons, Inc., New York, N. Y., 1946, p. 148.

(13) C. S. Marvel and R. D. Rands, THIS JOURNAL, **72**, 2642 (1950).

(14) E. P. Clarke, "Semimicro Quantitative Organic Analysis," Academic Press, Inc., New York, N. Y., 1943, p. 85.

chloride was added. After standing overnight the precipitate was washed with hot water and then dissolved with dilute sulfuric acid. Titration with potassium permanganate indicated the presence of 19.1 mg. of oxalic acid in the entire sample, or 0.54 mole per mole of dibasic acid. This amount is, of course, low owing to the loss by sublimation mentioned above.

Another aliquot was chromatographed on silicic acid according to the method of Marvel and Rands.[13] However, difficulty was encountered in dissolving the sample for placement on the column in the manner recommended, and only part of the added acid was recovered. The zone containing most of the acidity was eluted while the column was being developed with 25% butanol in chloroform. This zone was made definitely alkaline with sodium hydroxide and the chloroform layer discarded. The remaining aqueous layer was evaporated to about 10 ml. and then acidified to *p*H 2.0 with dilute sulfuric acid. The solution was extracted continuously with ether for 24 hours. The aqueous extractive was concentrated to a small volume under reduced pressure, and from this solution an acid crystallized. After several recrystallizations from water 9 mg. of a nitrogen-containing acid was obtained. The compound sintered and rolled about 185° and then melted at 225–230° in a sealed capillary tube. When the same sealed melting-point capillary tube was partially immersed in a bath at 200°, a sublimate that melted sharply at 234° deposited in the upper cooler part of the sealed tube. A potentiometric titration curve of the acid was typical of a dibasic acid and the neutralization equivalent was 87. These data indicate that the compound might be quinolinic acid, the sublimate in the melting point tube being the decarboxylated acid, that is, nicotinic acid m.p. 234°. A sample of quinolinic acid exhibited the identical behavior described above. Both quinolinic and the isolated acid gave an orange color with ferrous sulfate, and the ultraviolet spectra of the free acids and their sodium salts were identical. The acid, which was dried at 80° under high vacuum (oil-pump), gave an elemental analysis[15] that was in good agreement with that calculated for quinolinic acid.

Anal. Calcd. for $C_7H_5O_4N$; C, 50.30; H, 3.02; N, 8.38. Found; C, 50.50; H, 3.27; N, 8.8.

In another experiment some of the non-volatile acidic residue was chromatographed on paper with solvent A as described above for the dibasic acids. The paper was cut up into sections 1 cm. long. Each section was then eluted with 3 ml. of hot methanol, and the absorbancy of the solution was read in a Beckman spectrophotometer at room temperature. Only one zone that absorbed in the ultraviolet region was found. There was some absorbancy at the solvent front, but this absorbancy was also found at the same point in a blank run in exactly the same manner.

In the oxidation of the wilfordine dibasic acid, to be described in the next section, 1.07 moles of acetic acid was isolated from the oxidation mixture. This high yield of acetic acid (theory, 1.00 mole) necessitated a review of the oxidation procedure. It was found that in the neutralization of the dibasic acid prior to oxidation a small drop of phenolphthalein in 95% ethanol was added. The ethanol was oxidized to acetic acid in the subsequent oxidation and therefore gave a high yield of acetic acid. The oxidation was repeated on a 10-mg. sample of the dibasic acid by heating the neutralized acid with excess permanganate in a boiling water-bath for three hours while being vigorously stirred. No indicator was added; instead a *p*H meter was employed in neutralizing the acid. After the treatment as described above, approximately 0.2 mole of acetic acid was still found. The presence of acetic acid indicates that the compound must contain a methyl group on the side chain.

Oxidation of the Dibasic Acid of Wilfordine and Identification of the Products.—A solution of 86.3 mg. of dibasic acid of wilfordine in 25 ml. of water was oxidized exactly as described above for the dibasic acid of wilforine. The solution, however, was decolorized by dropwise addition of hydrogen peroxide and then heated to dispel excess peroxide. As above the steam-volatile acid fraction was chromatographed. Only one zone was obtained, and it contained 1.07 moles of acid per mole of dibasic acid. It had the same threshold volume as acetic acid, and gave the following steam distillation data: 17 ml., 17.3% of the acid; 34 ml., 31.8; 51 ml., 44.9; 68 ml., 55.6. From a comparison of these data with those obtained from the known acetic acid (see above), the acid was identified as acetic.

The continuous ether extract of the acidified non-volatile residue required 13.40 ml. of 0.0948 *N* alkali for neutralization, or 3.90 equivalents per mole of dibasic acid. It was determined as above that 27.9 mg. of oxalic acid, or 0.86 mole of acid per mole of dibasic acid, was present in this extract. Quinolinic acid was also separated (total amount unknown) and identified by melting point, ultraviolet absorption spectra of the free acid and its sodium salt, neutralization equivalent, and by the color reaction with ferrous sulfate. It also had the same threshold volume as quinolinic acid on the chromatographic column.

As described in the previous section, the oxidation of this dibasic acid was repeated on a 10-mg. sample without the addition of indicator solution. Again approximately 0.2 mole of acetic acid was found.

In subsequent experiments it was found that quinolinic acid could be obtained directly from the dry oxidation acid mixture, without resorting to separation by chromatography, by extraction with methanol and crystallizing the extractive from water.

Decarboxylation of the Dibasic Acids.—The dibasic acids were decarboxylated by heating at 240° in quinoline in the presence of basic cupric carbonate.[16] Each mole of the wilforine dibasic acid gave 0.99 mole of carbon dioxide upon decarboxylation, whereas a mole of the wilfordine dibasic acid gave 1.94 moles of carbon dioxide. The amount of carbon dioxide evolved after two hours was weighed. This weight was not increased in subsequent weighings. The reaction was therefore complete.

Polyhydroxy Nuclei of the Alkaloids.—From the degradation of the 1.05-g. lot of wilforine the hot methanol extract containing the polyhydroxy compound weighed 0.442 g. After three crystallizations pure white crystals that weighed 0.295 g. were obtained; yield 66%. This and the other three polyhydroxy compounds were dried at 80° under high vacuum (oil-pump) for analysis.

Anal. Calcd. for $C_{15}H_{26}O_{10}$: C, 49.18; H, 7.15. Found: C, 49.03; H, 7.02.

In the same manner the polyhydroxy compounds were isolated from wilfordine (C, 49.33; H, 7.11), wilforgine (C, 49.31; H, 7.13), and wilfortrine (C, 49.30; H, 7.21).

Paper Chromatography of the Polyhydroxy Nuclei.—The polyhydroxy compounds were chromatographed on strips of paper as described above for the dibasic acids except that the strips were not washed before the run was made. The following solvents were employed: (A) water-saturated phenol; (B) butanol, 4 ml.; acetic acid, 1 ml.; water, 5 ml., upper layer used; (C) water-saturated mixed collidines; Eastman Kodak T4815.

These solvents are the same as those used by Partridge[17] for the separation of sugars, except that the water-rich phase was not used to saturate the atmosphere. After development (16–20 hours) the strips were thoroughly washed with ether to remove the developing solvent and allowed to dry. The strips were cut into sections 1 cm. long, extracted with hot methanol and the polyhydroxy compound was detected in the residue after removal of methanol by rapidly heating with a small amount of concentrated sulfuric acid. The sulfuric acid remains clear in the absence of polyhydroxy compound but blackens to an extent that depends roughly on the amount of polyhydroxy compound present. A definite positive test was obtained with as little as 10 μg. of the compound, about 75 μg. usually being placed on each strip. A blank strip was always run, and this strip as well as all the other strips showed a weak band near the solvent front due to an impurity in the paper. The polyhydroxy compound was usually found to be spread across three sections. The data ($R_F \times 100$) summarized below show no significant difference in the chromatography of the four polyhydroxy nuclei from each alkaloid with the three solvents

	A	B	C
Wilforine	68.5	35	70
Wilfordine	69.5	34	72
Wilforgine	71	34	71
Wilfortrine	69	36	70

(15) Analysis by Schwartzkopf Microanalytical Laboratory, Middle Village, N. Y.

(16) Modification of the method of M. H. Hubacher, *Anal. Chem.*, **21**, 945 (1949).

(17) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

X-Ray Diffraction Patterns of the Polyhydroxy Nuclei.—The data from the X-ray diffraction patterns are summarized in Table II. There is no significant difference in the results obtained from the polyhydroxy compounds of the four alkaloids.

TABLE II

d/n VALUES[a] AND INTENSITY OF LINES[a] FROM X-RAY DIFFRACTION PATTERNS OF POLYHYDROXY NUCLEI FROM THE VARIOUS ALKALOIDS

Wilforine	Wilfordine	Wilforgine	Wilfortrine
12.10 vw	12.10 vw	11.93 vw	11.93 vw
8.10 s	8.10 s	8.10 s	8.10 s
7.19 vw	7.19 vw	7.19 vw	7.13 vw
6.40 s	6.45 s	6.40 s	6.40 s
5.90 m	5.94 m	5.94 m	5.90 m
5.09 w	5.09 w	5.06 w	5.06 w
4.43 vw	4.41 vw	4.41 vw	4.43 vw
4.11 vw	4.125 vw	4.125 vw	4.125 vw
3.69 vw	3.72 vw	3.72 vw	3.72 vw
3.41 w	3.41 w	3.40 w	3.41 w
3.00 vw	3.00 vw	2.99 vw	2.99 vw
2.81 w	2.82 w	2.82 w	2.82 w
2.68 vw	2.685 vw	2.68 vw	2.685 vw
2.43 vw	2.43 vw	2.43 vw	2.43 vw
2.24 mw	2.25 mw	2.24 mw	2.24 mw
2.03 w	2.035 w	2.03 w	2.03 w

[a] See Table I for explanation of symbols used in Table II.

Active Hydrogen Determinations on the Intact Alkaloid.—These determinations were made according to the directions of Pregl,[18] on 10-mg. samples. The reaction mixture was heated in boiling water to ensure completeness of the reaction. The results are summarized in Table III.

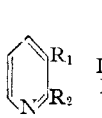
TABLE III

ACTIVE HYDROGEN DETERMINATIONS ON THE ALKALOIDS

Alkaloid	Solvent used in detn.	Found	Active hydrogen, % Theoretical 2 moles	3 moles
Wilforine	Anisole[19]	0.26	0.233	...
	Xylene	.248	.233	...
Wilfordine	Anisole[19]	.37	...	0.342
	Xylene	.367	...	.342
Wilforgine	Xylene	.244	0.235	...
Wilfortrine	Xylene	.323	...	0.346

Discussion

Alkaline permanganate oxidation of both dibasic acids yielded quinolinic, oxalic and acetic acids. These acids account for all of the eleven carbon atoms in the molecule. For the dibasic acid of wilforine (or wilforgine) six possible formulas may be written that will give the aforementioned three acids upon alkaline permanganate oxidation.



	R_1	R_2
I	COOH	$CH(C_2H_5)CH_2COOH$
II	$CH(C_2H_5)CH_2COOH$	COOH
III	COOH	$CH_2CH(C_2H_5)COOH$
IV	$CH_2CH(C_2H_5)COOH$	COOH
V	COOH	$CH_2CH(CH_3)CH_2COOH$
VI	$CH_2CH(CH_3)CH_2COOH$	COOH

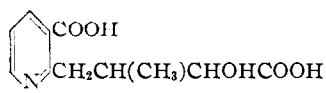
Formulas II, IV and VI were shown to be untenable, because the acid gives a negative test for a carboxyl group ortho to the pyridine nitrogen.[11]

(18) F. Pregl, "Quantitative Organic Microanalysis," P. Blakiston's Son and Co., Inc., New York, N. Y., 3rd English edition, 1937, p. 156.

(19) The author is grateful to W. C. Alford of the National Institutes of Health, Bethesda, Maryland, for making the determinations in anisole.

Of the three remaining formulas, V appears to be the most likely. Formulas I and III have ethyl groups on the side chain of the pyridine nucleus, and such groups are seldom found in natural compounds, whereas methyl groups, such as shown in formula V, are common.

The close relationship between the dibasic acid of wilforine and that of wilfordine is apparent from the almost identical ultraviolet absorption spectra of these acids and of their sodium salts (Figs. 1 and 2). Since the empirical formulas of these compounds differ by one oxygen, the dibasic acid of wilfordine (or wilfortrine) appears to be the hydroxy congener of the wilforine (or wilforgine) dibasic acid. In line with this view analyses on the intact alkaloids show one more active hydrogen to be present in wilfordine and wilfortrine than in wilforine and wilforgine (see Table III). The volatile acids contain no active hydrogens when esterified in the intact alkaloid, and it has been shown that aside from the dibasic acid the only other fragment, the polyhydroxy nucleus, is identical in all four alkaloids. Therefore, the active hydrogen must be present in the dibasic acid of wilfordine and the one extra oxygen in this acid must be present as an hydroxyl group. Infrared absorption spectra of the whole alkaloids as Nujol mulls substantiate this belief. The spectra of wilfordine and wilfortrine show peaks in the hydroxyl region (2.83 μ) that are absent in the spectra of wilforine and wilforgine.[20] From these data it seems most likely that the hydroxyl group of the dibasic acid is not esterified in the intact alkaloid. Further substantiation of the presence of an hydroxyl group may be drawn from gross observations on the solubility of the alkaloids in non-polar solvents. Wilfordine and wilfortrine are less soluble than wilforine and wilforgine, respectively, in ether, carbon disulfide and carbon tetrachloride. One would expect the alkaloids with the extra hydroxyl group to have a lesser solubility in non-polar solvents if the hydroxyl group were the only point of difference between the two sets of alkaloids, which it apparently is. Finally, the dibasic acid of wilfordine gives a positive test for an α-hydroxy acid,[12] a test that is negative for the dibasic acid of wilforine. This result establishes the position of the hydroxyl group in the dibasic acid. The hydroxy dibasic acid is therefore believed to have the formula



After the probable formulas for the dibasic acids had been determined, it was realized that these acids could be decarboxylated. Hubacher[16] has described a method and apparatus for splitting off and determining quantitatively the carboxy groups attached to an aromatic nucleus. The method also determines the carboxy group in hydroxy acids. A modification of this method was employed. Upon decarboxylation each mole of wilforine dibasic acid yielded one mole of carbon dioxide. This result is in line with the probable

(20) Figure 5 of ref. 3 and Fig. 3 of ref. 4.

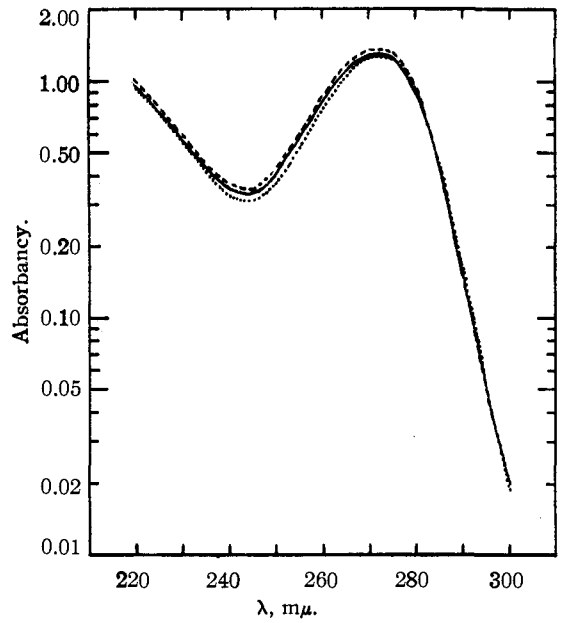


Fig. 1.—Ultraviolet absorption curves of dibasic acids in water, 0.050 mg./ml.; wilforine, dashed line; wilforgine, solid line; wilfordine and wilfortrine, dotted line.

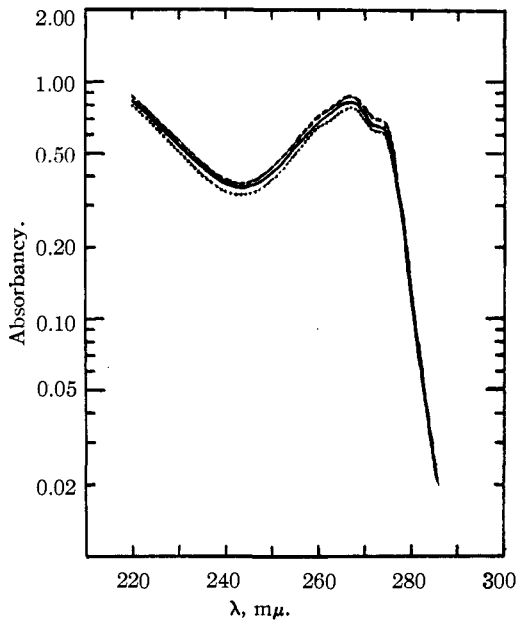


Fig. 2.—Ultraviolet absorption curves of neutralized dibasic acids in water, sodium salt of 0.0484 mg. acid/ml.: description of lines in Fig. 1.

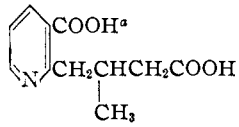
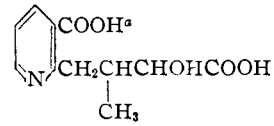
formula, which shows one carboxy group attached to the pyridine ring. Upon decarboxylation of the wilfordine dibasic acid, two moles of carbon dioxide were obtained per mole of acid—one from the carboxy group attached to the ring and one from the α-hydroxycarboxy group. Decarboxylation studies were therefore in line with the formulas presented.

None of the dibasic acids discussed above are known. It is hoped that it will be possible to synthesize either these compounds or a decarboxylated product of these acids to prove the structure of the two acids.

Wilforine, wilfordine, wilforgine and wilfortrine contain 2, 3, 2 and 3 moles of active hydrogen per mole of alkaloid, respectively. From these results it may be concluded that the polyhydroxy nucleus $C_{15}H_{26}O_{10}$ contains two unesterified hydroxyl groups (eight are esterified) and no indifferent oxygens. As previously mentioned, wilfordine and wilfortrine each have one active hydrogen on their dibasic acid, so that each of these entire alkaloids contains a total of three active hydrogens.

The four alkaloids account for about 90% of the total alkaloids in Acree and Haller's "wilfordine." The amount of each alkaloid in the mixture will vary, among other things, with the number of recrystallizations of the "wilfordine." A preparation crystallized several times was found to have wilforine, 25%; wilfordine, 44%; wilforgine, 13%; and wilfortrine, 7%.

Structure studies on the four alkaloids may be summarized by listing the fragments obtained from the saponification of each alkaloid as follows:

Wilforine	Wilforgine	Wilfordine	Wilfortrine
$C_{15}H_{16}(OH)_{10}$	$C_{15}H_{16}(OH)_{10}$	$C_{15}H_{16}(OH)_{10}$	$C_{15}H_{16}(OH)_{10}$
+	+	+	+
5 acetic acids	5 acetic acids	5 acetic acids	5 acetic acids
+	+	+	+
benzoic acid	3-furoic acid	benzoic acid	3-furoic acid
+	+	+	+
			

Sum of Components			
$-8\ H_2O$	$C_{43}H_{49}NO_{18}$	$C_{41}H_{47}NO_{19}$	$C_{43}H_{49}NO_{19}$	$C_{41}H_{47}NO_{20}$
Molecular formula	$C_{43}H_{49}NO_{18}$	$CC_{41}H_{47}NO_{19}$	$C_{43}H_{41}NO_{19}$	$C_{41}H_{47}NO_{20}$

[a] Proposed formula.

BELTSVILLE, MD.