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

Alkaloids from *Tripterygium wilfordii* Hook. Isolation and Structure of Wilforzine

BY MORTON BEROZA

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A small quantity of a new alkaloid, designated wilforzine, has been isolated from the roots of *Tripterygium wilfordii* by partition chromatography employing ultraviolet absorbancy ratios. Upon saponification the alkaloid yields 4 moles of acetic acid, 1 mole of benzoic acid, 1 mole of a nitrogen-containing dicarboxylic acid and a polyhydroxy nucleus. The polyhydroxy compound is shown to be identical with the polyhydroxy compounds previously isolated from wilforine, wilfordine, wilforgine and wilfortrine. The dicarboxylic acid is identical with the one isolated from wilforine and wilforgine. The formula for the sum of the components of wilforzine is in agreement with the molecular formula $C_{41}H_{47}O_{17}N$, calculated from elementary analyses on the intact alkaloid. The alkaloid therefore appears to be identical with wilforine, except that it contains one less acetyl group. This conclusion is confirmed by the fact that acetylation of wilforzine produces wilforine as judged by the X-ray diffraction patterns of their crystals. Evidence that wilforzine is not an artifact is also presented.

Four insecticidal alkaloids—wilforine, wilfordine, wilforgine and wilfortrine—have been isolated from the roots of *Tripterygium wilfordii* Hook.[1,2] Structure studies[3] show that the four alkaloids contain the same polyhydroxy nucleus. This compound contains 10 hydroxyl groups, 8 of which are esterified in the intact alkaloid, 5 with acetic acid, 1 with either benzoic or 3-furoic acid, and 2 with a nitrogen-containing dicarboxylic acid. Wilforine and wilforgine have the same dicarboxylic acid, and wilfordine and wilfortrine have the same dicarboxylic acid, but the latter is the hydroxy congener of the former.

After the four alkaloids had been isolated from the methanol-insoluble fraction (same as Acree and Haller's "wilfordine"[4]) by partition chromatography employing ultraviolet absorbancy ratios,[5] attention was directed toward the isolation of alkaloids present in lesser amounts.

Because it was not realized during the early work on this problem that the methanol-insoluble fraction was a mixture, this fraction was recrystallized as many as ten times to eliminate impurities. Subsequently preparations were recrystallized only once or twice. When they were chromatographed, as previously described,[1,2] an appreciable amount of material followed wilforgine and preceded wilfordine off the columns. From this material there was isolated 70 mg. of a new alkaloid for which the name "wilforzine" is suggested.

When wilforzine was chromatographed it separated only partially from wilforgine, but the amount of each in the various fractions was readily estimated by following the ultraviolet absorbancy ratios at 270 and 255 mμ. The absorbancy ratio of wilforzine is 1.33 and of wilforgine, 0.96. It is therefore possible by rechromatographing several times to separate the two compounds into pure wilforgine, pure wilforzine, and a mixture of the two in the same manner as wilforgine was separated from wilforine.[2]

As in previous work, the purity of the compound was tested by countercurrent distribution between benzene–hexane and hydrochloric acid. The distribution pattern was in very good agreement with a superimposed theoretical curve.[6]

(1) M. Beroza, THIS JOURNAL, **73**, 3656 (1951).
(2) M. Beroza, *ibid.*, **74**, 1585 (1952).
(3) M. Beroza, *ibid.*, **75**, 44 (1953).
(4) F. Acree and H. L. Haller, *ibid.*, **72**, 1608 (1950).
(5) M. Beroza, *Anal. Chem.*, **22**, 1507 (1950).
(6) B. Williamson and L. C. Craig, *J. Biol. Chem.*, **168**, 687 (1947).

Wilforzine melts at 177–178° and does not contain methoxy, methylenedioxy or alkimide groups. Upon saponification wilforzine yields 7 equivalents of acid, of which 5 are steam-volatile and 2 are steam-non-volatile. The steam-volatile acids consist of 4 equivalents of acetic acid and 1 equivalent of benzoic acid. From the non-volatile acid fraction a dibasic acid was isolated. It was shown to be identical with the dicarboxylic acid isolated from both wilforine and wilforgine. The probable structural formula of this acid has been reported.[3]

The polyhydroxy nucleus of the alkaloid was also isolated, and it proved to be identical with the polyhydroxy nucleus of wilforine, wilfordine, wilforgine and wilfortrine.

All the fragments resulting from saponification of wilforzine were therefore recovered and the formula for the sum of the component parts is in agreement with the formula $C_{41}H_{47}O_{17}N$, calculated from elementary analyses on the intact alkaloid.

Wilforzine yields the same products as wilforine upon saponification, except that it yields one less acetic acid. The infrared and ultraviolet spectra of wilforine and wilforzine are very similar. It is therefore likely that an acetyl group is the only point of difference between the two compounds. The conversion of wilforzine to wilforine by acetylation would confirm the foregoing structural studies and was therefore carried out. The product was shown by countercurrent distribution to contain no wilforzine after acetylation. The X-ray diffraction patterns of wilforine and acetylated wilforzine were prepared and proved to be identical.

The possibility exists that wilforzine is an artifact. Another preparation of the methanol-insoluble fraction was carefully worked up, and again wilforzine was isolated. There was no evidence of decomposition of wilforine to wilforzine in the chromatographic separation procedure, but it is recognized that the presence of methanol in the crystallizing solvent of wilforine could produce an ester-interchange reaction and thus yield wilforzine and methyl acetate. Such an interchange would be expected to take place in the presence of acid or alkali. Since wilforine is very feebly alkaline, it does not seem likely that such an interchange would occur, particularly in the cold (crystallized in ice-box). The wilforine crystallizes out of solution very rapidly so that it constitutes a separate phase, and in this condition ester-interchange appears even less likely. If the interchange were

taking place, probably the same acetyl group would not always be split off and more than one kind of crystal would form. The crystals in a mounting liquid were examined under a polarizing microscope, but no inhomogeneity of crystal structure could be detected.

To determine further whether ester-interchange was taking place, wilforine was allowed to remain in methanol for five days at room temperature. Countercurrent distribution study showed that no wilforzine was formed. Experimental data therefore indicate that wilforzine is not an artifact.

Wilforzine was much less toxic to diamondback moth larvae than wilforine. This result indicates that insecticidal activity of the *Tripterygium* alkaloids may depend upon the ester groups being intact.

Experimental[7]

Isolation of Wilforzine.—The partition chromatographic columns were prepared and operated as already described.[1]

Material from several preparations that was eluted after wilforgine and before wilfordine was combined and chromatographed with 2% hydrochloric acid as the immobile solvent and ether as the mobile solvent. A zone containing appreciable material separated incompletely from the wilforgine and other alkaloids present in this fraction. After this zone had been chromatographed three times and the ends of the zone removed each time, the material showed a constant ultraviolet absorbancy ratio of 1.33 ± 0.01. The solvent was removed, a minimum of acetone was added to dissolve the residue and, after the addition of a small amount of absolute methanol, wilforzine crystallized out as needle-like crystals, m.p. 177–178°, $[\alpha]^{25}D$ +6 ± 2° (acetone); yield about 1–2% based on methanol-insoluble fraction. For analysis the compound was dried at 56° under high vacuum.

Anal. Calcd. for $C_{41}H_{47}O_{17}N$: C, 59.61; H, 5.74; N, 1.7; mol. wt., 826. Found: C, 60.07; H, 5.93; N, 1.6; mol. wt., 824.

The ultraviolet absorption spectra of wilforzine both in absolute ethanol [λ_{max} 269 mμ (log ε 3.62), 229 mμ (log ε 4.29) and λ_{min} 253 mμ (log ε 3.45)] and in 1% hydrochloric acid [λ_{max} 268 mμ (log ε 3.91), 231 mμ (log ε 4.25) and λ_{min} 254 mμ (log ε 3.71)] are almost identical with those of wilforine and wilfordine in the two respective solvents from 220 to 300 mμ. Such a result was expected, since these compounds contain the same chromophoric groups. The infrared spectra[8] in carbon tetrachloride and as a Nujol mull possess the same principal maxima as the corresponding spectra of wilforine,[1] thereby demonstrating the similarity of the chemical structure of these compounds. The main difference between the spectra is in the Nujol spectrum of wilforzine; the hydroxyl peak (2.95 μ) is much more prominent than the corresponding peak in the spectrum of wilforine. The one less acetyl group in wilforzine, which leaves an extra hydroxyl group free, would be expected to give such a result.

The solubility properties of wilforzine are similar to the properties of the four alkaloids previously isolated, except that wilforzine has a greater solubility in methanol and in ethanol.

Countercurrent Distribution.—Distributions were carried out manually with a special transfer syringe.[9] A solution containing 30% hexane in benzene (v./v.) and 2% hydrochloric acid were used to perform a 20-plate distribution of wilforzine. The pattern was in very good agreement with a theoretical one for K (org./aq.) = 0.8. Analyses of the alkaloid in each tube were carried out as previously described.[1]

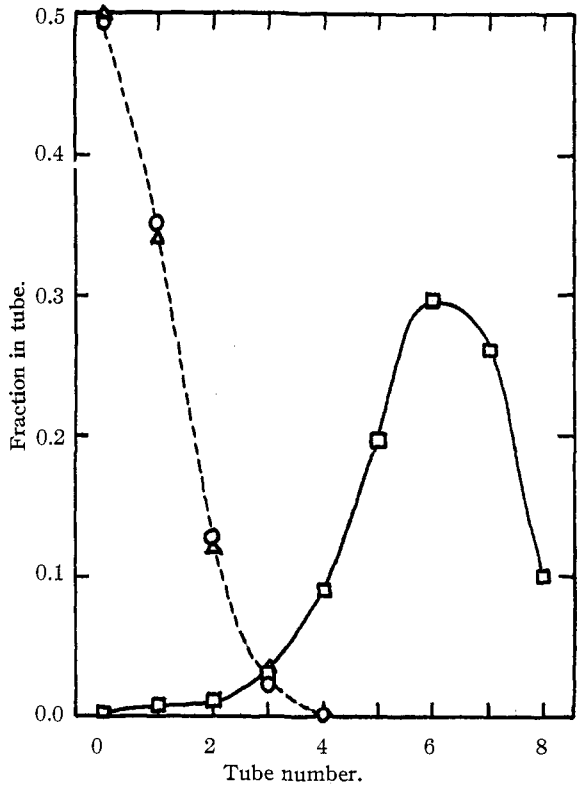

Fig. 1.—Countercurrent distribution patterns, 40% hexane in benzene–2% HCl: solid line, wilforzine; dotted line with triangles, acetylated wilforzine; dotted line with circles, wilforine after 5 days in methanol.

Saponification of Wilforzine.—8.637 and 28.00 mg. of wilforzine were each saponified with diethylene glycol–potassium hydroxide reagent.[10] The respective saponification equivalents were determined to be 118.7 and 119.0 (6.96 and 6.94 equivalents of acid per mole of alkaloid).

Acids in Wilforzine.—The titrated solution from the 8.637-mg. sample of wilforzine was acidified to congo red with sulfuric acid and steam distilled exhaustively. The distillate contained 5.07 equivalents of steam-volatile acid per mole of alkaloid. Chromatography of these acids[11] on a 10-g. column gave two zones. The first zone eluted contained 0.94 equivalent of acid and the second, 3.95 equivalents. The continuous ether extract of the steam non-volatile residue (at *p*H 2.8) contained 1.83 equivalents of acid per mole of alkaloid.

The titrated solution from the 28.00-mg. sample was treated as described in the outline of operations.[3] Chromatography of the steam-volatile acids gave 0.80 equivalent of acid in the first zone eluted and 4.05 equivalents in the second zone. The acid in the first zone was identified as benzoic by its elution volume, ultraviolet spectrum between 220 and 300 mμ, and its semidistillation value of 18% (literature[12] 17.1%). The second zone was identified as acetic by its elution volume and by Duclaux numbers: known acetic acid: 6.3, 6.5, 6.6; found: 6.3, 6.3, 6.6.

The steam-non-volatile extractive contained 1.83 equivalents of acid per mole of alkaloid. From this extractive the dibasic acid of wilforzine was isolated. It melted at 192–195°, and in admixture with the dibasic acid from wilforgine (m.p. 195°) it melted at 193–195°. The acid was purified by taking up in a minimum of acetone, adding chloroform,

(7) All melting points are corrected. Determinations of carbon, hydrogen and nitrogen were made by the Oakwold Laboratory, Alexandria, Va. All ultraviolet spectrophotometric measurements were made on a Beckman model DU quartz spectrophotometer. X-Ray diffraction patterns were prepared and microscopic examination of crystals was made by E. L. Gooden, of this Bureau. Tests for insecticidal activity were made by G. T. Bottger, of this Bureau.

(8) The author is grateful to Jonas Carol, of the U. S. Food and Drug Administration, for preparing these spectra.

(9) M. Beroza, *Anal. Chem.*, **23**, 1055 (1951).

(10) Schneider, "Qualitative Organic Microanalyses," John Wiley and Sons, Inc., New York, N. Y., 1946, p. 161.

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

(12) A. A. Morton, "Laboratory Technique in Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1938, p. 128.

and allowing it to crystallize. In previous work 95% ethanol was the crystallizing solvent, but yields were lower.

The acid was further shown to be identical with the wilforine dibasic acid on the basis of X-ray diffraction data, ultraviolet spectra of the free and neutralized acid, and paper chromatography with three different solvents as previously described.[3] The results ($R_f \times 100$) of the chromatography of the dibasic acids follow:

	A	B	C
Wilforine	92	90.5	87
Wilforzine	92	89.5	85.5

Polyhydroxy Nucleus of Wilforzine.—From the 28.00-mg. lot of wilforzine the polyhydroxy nucleus was isolated.[3] Its X-ray diffraction pattern was indistinguishable from the patterns of the polyhydroxy nuclei of wilforine, wilforgine, wilfordine and wilfortrine. Paper chromatography of the compound run simultaneously with the polyhydroxy nucleus from wilforine as described[3] showed no significant difference in results ($R_f \times 100$) with the three different solvents:

	A	B	C
Wilforine	69	37	72
Wilforzine	68	36	72

Acetylation Studies.—To 4 mg. of wilforzine was added 0.5 ml. of acetic anhydride and 0.5 ml. of pyridine. After remaining at room temperature for 5 days the solution was evaporated to dryness on a 60° water-bath under reduced pressure. The resulting material was subjected to an 8-plate countercurrent distribution between a solution containing 40% hexane in benzene (v./v.) and 2% hydrochloric acid (Fig. 1). The distribution pattern when compared with that obtained from untreated wilforzine shows that no wilforzine remains after acetylation. The acetylated wilforzine, recovered from the countercurrent distribution, crystallized upon the addition of a few drops of methanol. An X-ray diffraction pattern of the crystals was identical with that obtained from crystals of wilforine. The *d/n* values (interplanar spacing in ångström units/order of diffraction) and intensity of lines (s = strong, m = medium, w = weak, v = very) obtained from both patterns are: 12.7 m, 10.3 vs, 8.0 s, 6.5 m, 5.9 vw, 4.85 s, 4.3 w, 3.7 w, 3.31 vw, 3.09 vw.

Attempted Methanolysis of Wilforine.—Wilforine was allowed to remain in contact with methanol for five days at room temperature, after which the product was tested for wilforzine by countercurrent distribution between a solution containing 40% hexane in benzene and 2% hydrochloric acid. None was found (see Fig. 1). The patterns of wilforine and acetylated wilforzine practically coincide and thereby constitute a further proof that the two compounds are identical.

BELTSVILLE, MD.

[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

The Structure of Silicicolin[1a]

BY JONATHAN L. HARTWELL, ANTHONY W. SCHRECKER AND JAMES M. JOHNSON

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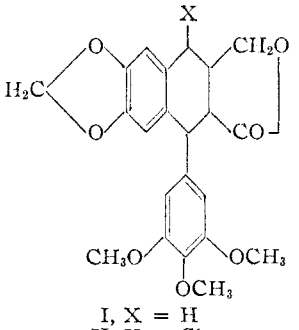
Silicicolin, obtained from the needles of *Juniperus silicicola*, has been prepared by hydrogenolysis of podophyllotoxin chloride, and is identical with desoxypodophyllotoxin.

In an earlier paper,[1b] silicicolin, a tumor-damaging substance obtained from the dried needles of *Juniperus silicicola* (Small) Bailey (Fam. *Pinaceae*) (Southern red cedar), had been characterized and tentatively assumed to be a lignan, perhaps the hitherto unknown desoxypodophyllotoxin (I). This assumption has now been shown to be correct. When podophyllotoxin chloride (II), prepared[2] from podophyllotoxin (III), was submitted to hydrogenolysis under the conditions of the Rosenmund reaction,[3] a 99% yield of desoxypodophyllotoxin, m.p. 168–169°, was obtained identical in all respects with the natural product[4] (Table I). Both the natural and synthetic compound could be epimerized to silicicolin-B (desoxypicropodophyllin), m.p. 169–173° and 170.5–172°, respectively, by heating with alcoholic solutions of sodium acetate or of piperidine, while both gave the same hydroxy acid (desoxypodophyllic acid) on saponification.



I, X = H
II, X = Cl
III, X = OH

The literature contains references to (a) two different "desoxypicropodophyllins," prepared by hydrogenation of α-[5] and of β-apopicropodophyllin,[6] respectively; (b) a natural product, anthricin,[7] isolated from the roots of *Anthriscus sylvestris* Hoffm. (Fam. *Umbelliferae*) (wild chervil), which has also been assigned structure I and can be epimerized to isoanthricin; and (c) another natural product, cicutin,[8] separated from the roots of *Cicuta maculata* L. (Fam. *Umbelliferae*), (water hemlock), about whose structure all that is known is that it has the empirical formula $C_{22}H_{22}O_7$ and contains three methoxyl groups and a lactone ring. Table I gives comparative physical constants on all the lactones mentioned here. Since all but one of the compounds listed has a melting

(1) (a) Presented before the Division of Medicinal Chemistry at the 123rd National Meeting of the American Chemical Society, Los Angeles, Calif., March 15–19, 1953; (b) J. L. Hartwell, J. M. Johnson, D. B. Fitzgerald and M. Belkin, THIS JOURNAL, **74**, 4470 (1952).

(2) J. L. Hartwell and A. W. Schrecker, *ibid.*, **73**, 2909 (1951).

(3) E. B. Hershberg and J. Cason, *Org. Syntheses*, **21**, 84 (1941); R. P. Barnes, *ibid.*, **21**, 110 (1941).

(4) We have been unable to find any prior reference to this useful extension of the Rosenmund reaction to benzhydryl-type halides. The advantage of this technique lies in the fact that the course of the reaction can be followed readily by measuring the hydrogen chloride evolved.

(5) W. Borsche and J. Niemann, *Ann.*, **494**, 126 (1932); *cf.* A. W. Schrecker and J. L. Hartwell, THIS JOURNAL, **74**, 5676 (1952).

(6) N. L. Drake and E. H. Price, *ibid.*, **73**, 201 (1951).

(7) K. Noguchi and M. Kawanami, *J. Pharm. Soc. Japan*, **60**, 629 (1940).

(8) L. Marion, *Can. J. Research*, **20B**, 157 (1942).