was identical with that of the hydrated sample except that it lacked the two peaks due to water.

Anal. Calcd. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found: C, 74.50; H, 9.78.

17α-Ethyl-3α,5α-cycloandrostane-6β,17β,19-triol.—A solution of 250 mg. of III in 10 ml. of purified tetrahydrofuran containing 0.60 g. of lithium acetylide–ethylenediamine 1:1 complex was stirred at room temperature for 2 hr. Addition of water gave the product as an oil which crystallized. Several recrystallizations from acetone–hexane gave 84 mg. of almost pure 17α-ethynyl-3α,5α-cycloandrostane-6β,17β,19-triol, melting at 225–235°.

Reduction at room temperature of 55.87 mg. of the ethynyl compound in 4 ml. of 95% ethanol was catalyzed by 6.02 mg. of 5% palladium on charcoal. After 0.5 hr. the hydrogen uptake was 8.00 ml. (theory, 8.03 ml.). Addition of water gave 50 mg. of 17α-ethyl-3α,5α-cycloandrostane-6β,17β-19-triol, m.p. 247–251°. The analytical sample, crystallized from acetone–hexane, had m.p. 248–253°; λ_{max}^{KBr} 2.80, 2.98, 3.13, 6.79, 9.36, 9.52, 9.67, and 9.86 μ.

Anal. Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.02; H, 10.03.

Acknowledgment.—The author wishes to thank Dr. Kurt Rorig for helpful discussions and encouragement, and Dr. Roy H. Bible for assistance with n.m.r. interpretations.

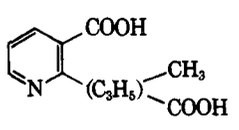
Alkaloids from *Tripterygium wilfordii* Hook. The Chemical Structure of Wilfordic and Hydroxywilfordic Acids

MORTON BEROZA

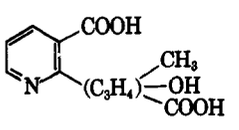
Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland

Received April 24, 1963

Alkaline saponification of five large insecticidal alkaloids (molecular weights between 826 and 891) isolated from the roots of the perennial twining vine *Tripterygium wilfordii* Hook yielded among other products two pyridine dicarboxylic acids whose structures were not completely identified. One of these ($C_{11}H_{13}NO_4$, m.p. 195–196°), for which the name wilfordic acid is proposed, was obtained from the alkaloids wilforine, wilforgine, and wilforzine.[1] The other ($C_{11}H_{13}NO_5$, m.p. 178–179°), for which the name hydroxywilfordic acid is suggested, was isolated from wilfordine and wilfortrine.[1] The following partial formulas summarize the information on the chemical structures of the acids as established previously by the author.[2]



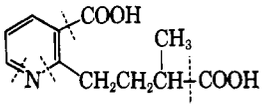
I, wilfordic acid



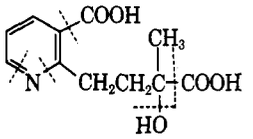
II, hydroxywilfordic acid

These formulas were based on elemental analyses, neutral equivalents, potentiometric titration, and the following data. Permanganate oxidation of both acids gave acetic, oxalic, and quinolinic acids; both acids failed to chelate with ferrous sulfate, indicating there is no carboxyl group *ortho* to the pyridine nitrogen atom. The hydroxyl group of hydroxywilfordic acid was shown to be alpha to a carboxyl group. Decarboxylation of the dibasic acids in quinoline in the presence of basic copper carbonate gave one mole of carbon dioxide for wilfordic acid and two for hydroxywilfordic acid.[3] It was concluded that a methyl group was present in the side chain, since acetic acid was present among the oxidation products.

This note shows that wilfordic acid is 3-carboxy-α-methyl-2-pyridinebutyric acid (III) and that hydroxywilfordic acid is 3-carboxy-α-hydroxy-α-methyl-2-pyridinebutyric acid (IV) (dotted lines of III and IV will be discussed in later section).

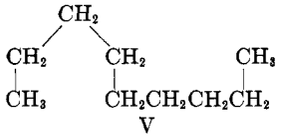


III, Wilfordic acid



IV, hydroxywilfordic acid

Both acids were subjected to analysis by the hydrogenolytic gas chromatography technique described by the author,[4] and each gave nonane (V, written so as to facilitate comparison with III and IV).



V

This chromatographic procedure would be expected to cleave the bonds of III and IV that are severed with dotted lines (for example, α-picoline gives hexane, RCOOH gives RH, and RR'CHOH gives RR'CH_2). Although the position of the methyl group is established from the nonane carbon skeleton, the positions of the hydroxyl and the carboxyl groups are not, since the carboxyl and hydroxyl groups could be attached to the carbons alpha and beta to the ring, and nonane would still be obtained.

Nuclear magnetic resonance spectra of the acids (Fig. 1 and 2) showed III and IV to be the correct structures. (Chemical shift data are given in parts per million from the water peak, taking the high field as positive.) In the wilfordic acid spectrum the methyl peak is a doublet at +3.59 ($J_{\alpha\text{-Me}}$ = 7 c.p.s.), split by a single proton (alpha to the carboxyl group) that appears as a multiplet at +2.15 p.p.m. Were the carboxyl alpha or beta to the ring, the methyl would be a triplet and not a doublet. A sample of 2-methylpentanoic acid run under the same conditions gave the same $J_{\alpha\text{-Me}}$ spin-coupling constant, and the chemical shifts for the peaks of the methyl and α-protons agreed closely (+3.66 and +2.33 p.p.m.) with the shifts of the corresponding structures in wilfordic acid. Splittings of the protons on the β-carbon were not readily resolvable, but those on the γ-carbon (adjacent to the ring) are a triplet at +1.48 ($J_{B\gamma}$ = 5.3 c.p.s.). The protons on the γ-carbon are expected to be furthest downfield since phenyl substituents cause a much larger paramagnetic shift of alkyl frequencies than a carbonyl double bond, and the heteroatom of the ring would be expected to accentuate

(1) M. Beroza, *J. Am. Chem. Soc.*, **73**, 3656 (1951); **74**, 1585 (1952); **75**, 2136 (1953).

(2) M. Beroza, *ibid.*, **75**, 44 (1953).

(3) Aromatic carboxylic and α-hydroxycarboxylic acids give one mole of carbon dioxide per mole of carboxyl group by the procedure of M. Beroza, *Anal. Chem.*, **25**, 177 (1953).

(4) M. Beroza, *ibid.*, **34**, 1801 (1962); **35**, 1353 (1963).

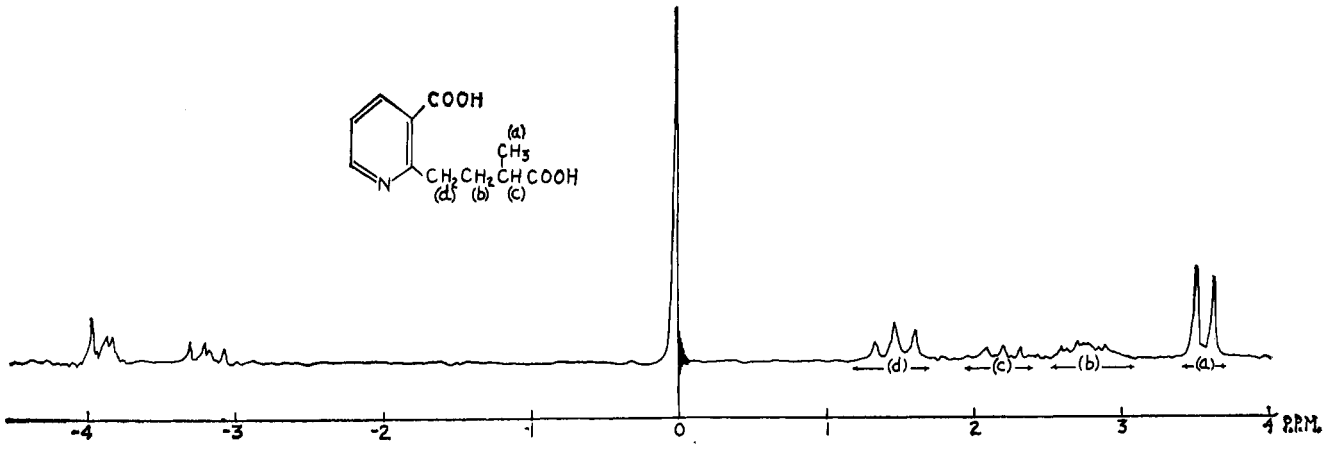


Fig. 1.—Nuclear magnetic resonance spectrum of wilfordic acid in deuteriowater.

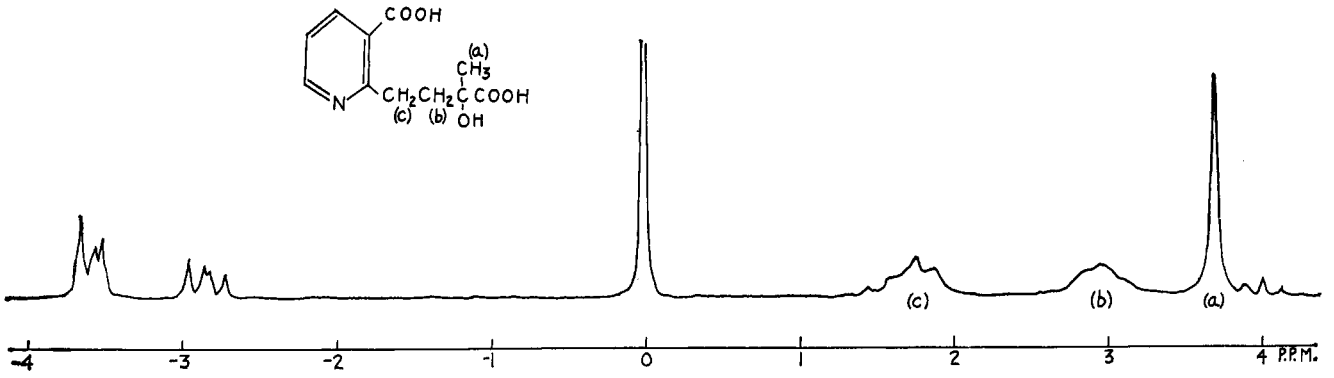


Fig. 2.—Nuclear magnetic resonance spectrum of hydroxywilfordic acid in deuteriowater.

this effect.[5] The integrated intensities of the peaks for H_{Me}, H_α, H_β, H_γ (3:1:2:2) are consistent with structure III. No side chain other than the one in III can satisfy the foregoing n.m.r. data.

Only 2 mg. of hydroxywilfordic acid was available and its spectrum was too poor to determine coupling constants. However, a singlet at +3.71 equivalent to three protons is clearly due to the methyl group known to be present. Since it is not split, both the carboxyl and the hydroxyl group must be attached to the carbon atom holding the methyl group. The remaining $-CH_2CH_2-$ of the side chain gives the expected two triplets (at +1.76 and +2.97) coupled with each other. The triplets, each equivalent to two protons, exhibit the characteristic coupling pattern in which the inner peaks increase at the expense of the outer ones.[6] These data are consistent only with structure IV. [The three small peaks above (a) of Fig. 2 are equivalent to much less than a proton and, therefore, are an impurity.]

The spectra of both acids were practically identical in the aromatic proton region (−3 to −4 p.p.m. from the H_2O peak), each displaying two groups of multiplets with integrated intensities equivalent to three protons. Protons on the carboxyl group are not visible in deuteriowater solutions.

Both acids are optically active. The asymmetric carbon atom in each is alpha to the carboxyl group of the side chain.

By alkaline saponification of evonine, the main alkaloid of the seeds of *Euonymus europaeus* L., Pailer and Libiseller[7] isolated a dibasic acid, evoninic acid, and showed that the compound was very similar chemically to wilfordic acid with which it is isomeric. Both are β-pyridinecarboxylic acids substituted in the α-position with a C_4H_8COOH side chain, but evoninic acid contains a 2,3-dimethylpropionic acid in place of the 2-methylbutyric acid side chain of wilfordic acid.

The great similarity of the pyridine dibasic acids obtained from *E. europeae* and *T. wilfordii* is not unexpected since both plants belong to the same family (Celastraceae). It already has been shown that another member of Celastraceae, the North American bittersweet (*Celastrus scandens* L.), contains the same insecticidally inert pigment that is present in *T. wilfordii*.[8]

The n.m.r. spectrum of the $C_{15}H_{26}O_{10}$ polyhydroxy nucleus found in all five of the alkaloids isolated from *T. wilfordii*[1,2] shows singlets at +3.01 and +3.31, each equivalent to three protons (presumably methyl groups). Since no double bonds are present and the nucleus is known to contain ten hydroxyl groups,[2] the empirical formula indicates the nucleus has three rings. It is interesting to note that Pailer and Libiseller[7] found the polyhydroxy nucleus of evonine has the same empirical formula ($C_{15}H_{26}O_{10}$) as that from *T. wilfordii*.

Experimental

Hydrogenolytic Gas Chromatography.—The apparatus of ref. 4 was used. About 20 μg. of each of the dibasic acids was injected onto a catalyst, consisting of neutral 1% palladium on

(5) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, pp. 57 and 63.

(6) See ref. 5, p. 90.

(7) M. Pailer and R. Libiseller, *Monatsh. Chem.*, **93**, 403, 511 (1962).

(8) M. S. Schechter and H. L. Haller, *J. Am. Chem. Soc.*, **64**, 182 (1942), and references therein.

Gas Chrom P (wt./wt.), maintained at 300°. The analytical column was a $^3/_{16}$-in. o.d. copper column containing 5% squalane on acid-washed Chromosorb W maintained at 110°. Both acids gave appreciable amounts of only one peak at 11.4 min. This retention time coincided precisely with that of nonane.

Nuclear Magnetic Resonance Spectra.—These were obtained on a Varian Associates Model A-60 spectrometer. Deuteriowater was used as the solvent because the solubility of the acids in the commonly used deuteriochloroform is too low. The spectrum of hydroxywilfordic acid was made with microplugs supplied by Varian Associates.

Optical Rotation.—In water the optical rotation was $[\alpha]^{24}D$ +6.98° for wilfordic acid and $[\alpha]^{24}D$ −24.1° for hydroxywilfordic acid.

Acknowledgment.—The author thanks Mrs. K. S. Warren of the National Institutes of Health for determining the optical rotation of the acids and Mr. Don Hollis of Varian Associates for reviewing the interpretation of the n.m.r. spectra.

Application of Nuclear Magnetic Resonance Spectroscopy to Keto Acid–Pseudoacid Tautomerism

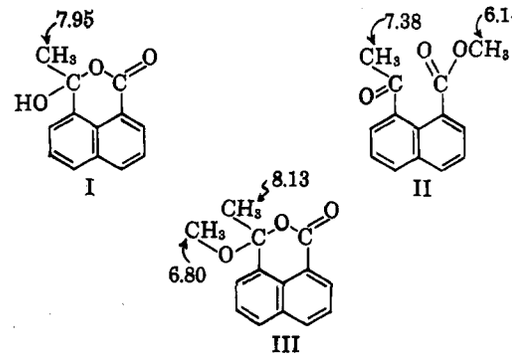
PETER T. LANSBURY AND JOSEPH F. BIERON[1]

Department of Chemistry, State University of New York at Buffalo, Buffalo, New York

Received July 15, 1963

Infrared[2] and ultraviolet[3] spectroscopy have been frequently used to study the structure as well as equilibrium composition of ring-chain tautomers of *o*-acylbenzoic and *o*-aroylbenzoic acids and their esters. Bonner[4] recently has made a detailed study of the normal and pseudo (ψ) methyl and (−)-menthyl esters of *o*-benzoylbenzoic acid (which is mainly in the open form[2a]), using not only infrared and ultraviolet spectroscopy for determining structure and detecting mixtures, but optical rotatory dispersion and thin layer chromatography as well. In connection with another problem, we have examined the nuclear magnetic resonance (n.m.r.) spectra of 8-acetyl-1-naphthoic acid and its normal and ψ-methyl esters, as well as the methyl *o*-benzoylbenzoates. This technique is extremely rapid and straightforward and may have general utility in the qualitative and quantitative analysis of normal and pseudoesters, particularly methyl esters, since unsplit methyl proton signals are easily seen and appear at different positions in the spectra, depending on which tautomer is present.

8-Acetyl-1-naphthoic acid (I) reportedly[5] does not give carbonyl derivatives, and its infrared spectrum further suggests that it exists as the pseudoacid. We have confirmed this by comparing the n.m.r. spectrum with the normal (II) and ψ- (III) methyl esters, which were prepared by treating I with diazomethane and acidified methanol, respectively. The chemical shifts (τ) of the various methyl protons are indicated. The



methyl signals in II are typical[6] for methyl aryl ketones and methyl benzoates, whereas the 6.80-τ signal in III is in the expected region for methyl ethers.[6] The 8.13-τ signal in III corresponds closely with the *single* methyl peak in I, thus confirming the ψ-acid character of I. Ultraviolet spectroscopy also shows that I exists in the ψ-acid form, as Table I indicates.

TABLE I

Compound	λ_{max} mμ	log ε
I	310	3.91
II	296	3.95
III	310	3.95

The quantitative analysis of mixtures of normal and ψ-esters can be accomplished readily by n.m.r. spectroscopy, using the peak areas of the individual methyl groups to calculate the ratio of the tautomers present. For example, a synthetic mixture containing 40.6% of II and 59.4% of III gave 41% II and 59% III by electronic integration on the A-60 spectrometer. Also, a synthetic mixture containing 52.7% of methyl *o*-benzoylbenzoate[7] (τ_{CH_3} 6.53) and 47.3% of methyl ψ-*o*-benzoylbenzoate[7] (τ_{CH_3} 6.82) contained 52% of the former and 48% of the latter by integration as above.

Experimental[8]

Pseudo Methyl Ester of 8-Acetyl-1-naphthoic Acid (III).—A solution of 5.6 g. of 8-acetyl-1-naphthoic acid[5] in 60 ml. of methanol containing a few drops of sulfuric acid was heated for 2 hr. on a steam bath. The reaction mixture was poured into water and extracted with ether. The ether was washed with 10% sodium hydroxide solution, water, and then dried over sodium sulfate. Evaporation of the ether, followed by recrystallization of the residue from methanol–water gave a quantitative yield (6.0 g.) of III, m.p. 102.5–103°, whose infrared spectrum (Nujol mull) showed a single carbonyl band at 1715 cm.$^{-1}$ (lactone).

Anal. Calcd. for $C_{14}H_{12}O_3$: C, 73.7; H, 5.3. Found: C, 74.2; H, 5.2.

Normal Methyl Ester of 8-Acetyl-1-naphthoic Acid (II).—An excess of ethereal diazomethane was added to 4.0 g. of 8-acetyl-1-naphthoic acid in ether, whereupon gas evolution began immediately. After 3 hr., the yellow solution was filtered and evaporated under nitrogen. The yellow crystalline residue was recrystallized from petroleum ether (b.p. 60–90°) to give II in quantitative yield, m.p. 92–93°. This ester showed infrared carbonyl bands (in Nujol) at 1660 (ketone) and 1725 cm.$^{-1}$ (ester).

Anal. Calcd. for $C_{14}H_{12}O_3$: C, 73.7; H, 5.3. Found: C, 73.6; H, 5.2.

(1) National Science Foundation predoctoral Fellow, 1962–1964

(2) (a) M. Renson, *Bull. soc. chim. Belges*, **70**, 77 (1961); (b) D. D. Wheeler, D. C. Young, and D. S. Earley, *J. Org. Chem.*, **22**, 547 (1957); (c) P. R. Jones and S. L. Congdon, *J. Am. Chem. Soc.*, **81**, 4291 (1959).

(3) M. S. Newman and C. W. Muth, *ibid.*, **73**, 4627 (1951).

(4) W. A. Bonner, *ibid.*, **85**, 439 (1963).

(5) P. R. Jones and A. A. Lavigne, *J. Org. Chem.*, **25**, 2020 (1960).

(6) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, p. 54.

(7) We thank Professor W. A. Bonner for samples of these compounds.

(8) Melting points were taken in a "Mel-temp" capillary tube melting point apparatus and are uncorrected. Elemental analyses were performed by Dr. Alfred Bernhardt, Mülheim, Germany.