30 min and was filtered through a 0.45-μm filter[7]. The charcoal pad was carefully removed from the filter and washed with 500 ml of distilled water to remove excess pyrophosphoric acid. The pteridine adsorbed on the charcoal pad was eluted by suspending the pad in 75 ml of 3 *N* ammonium hydroxide, and the mixture was stirred for 15 min. The resulting fine suspension was filtered through a fresh filter paper[7]. This procedure was repeated three times with the same quantity of 3 *N* ammonium hydroxide as eluant. The combined filtrate was evaporated under reduced pressure at room temperature until no odor of ammonia could be detected. The solution was lyophilized to yield about 300 mg (80%) of greenish-yellow amorphous powder. An analytical sample was obtained by recrystallization from aqueous ethanol, mp 166–175°; UV λ_{max} (0.1 *N* NaOH): 255 and 362 nm; IR ν_{max} (mineral oil): 3300, 1675 (NH_2), 1240 (P=O), 1040 (P—OH), and 820 (pteridine) cm^{-1}; NMR: δ 5.2 (m, 2H, CH_2) and 7.0 (d, 1H, aromatic H); R_f (water) 0.98.

Anal.—Calc. for $C_7H_{15}N_7O_8P_2$: C, 21.71; H, 3.90; N, 25.30; P, 15.99. Found: C, 21.86; H, 4.10; N, 24.50; P, 15.95.

[7] Millipore.

REFERENCES

(1) T. Shiota, *Arch. Biochem. Biophys.*, **80,** 155(1959).

(2) L. Jaenicke and P. C. Chan, *Angew. Chem.*, **72,** 752(1960).

(3) G. M. Brown, R. A. Weisman, and S. A. Molnar, *J. Biol. Chem.*, **236,** 2534(1961).

(4) T. Shiota, M. N. Disraely, and M. P. McCann, *Biochem. Biophys. Res. Commun.*, **7,** 194(1962).

(5) T. Shiota, M. N. Disraely, and M. P. McCann, *J. Biol. Chem.*, **239,** 2259(1964).

(6) L. Jaenicke and M. Silverman, *Deut. Med. Forsch.*, **1,** 24(1963).

(7) H. S. Forrest and J. Walker, *J. Chem. Soc.*, **1949,** 2077.

(8) S. Futterman, *J. Biol. Chem.*, **228,** 1031(1957).

(9) C. Levaditi and R. Perault, *C. R. Soc. Biol.*, **135,** 1043(1941).

(10) J. L. McCullough and T. H. Maren, *Antimicrob. Ag. Chemother.*, **3,** 665(1973).

(11) J. K. Seydel, *J. Pharm. Sci.*, **57,** 1455(1968).

(12) W. O. Foye and J. P. Speranza, *ibid.*, **59,** 259(1970).

(13) R. I. Ho, L. Corman, S. A. Morse, and M. S. Artenstein, *Antimicrob. Ag. Chemother.*, **5,** 388(1974).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 29, 1974, from the *Samuel M. Best Research Laboratory, Massachusetts College of Pharmacy, Boston, MA 02115*

Accepted for publication April 23, 1974.

Supported by Research Contract DAMD17-74-C-4039 from the U.S. Army Medical Research and Development Command.

The authors gratefully acknowledge the technical assistance of Johnna Ho and Por-Hsiung Lai.

[x] To whom inquiries should be directed.

Potential Anticancer Agents I: Confirming Evidence for the Structure of Fagaronine

M. TIN-WA, C. L. BELL, C. BEVELLE, H. H. S. FONG, and N. R. FARNSWORTH[x]

Abstract □ The structure of fagaronine was confirmed by spectral studies on its *N*-demethyl derivative.

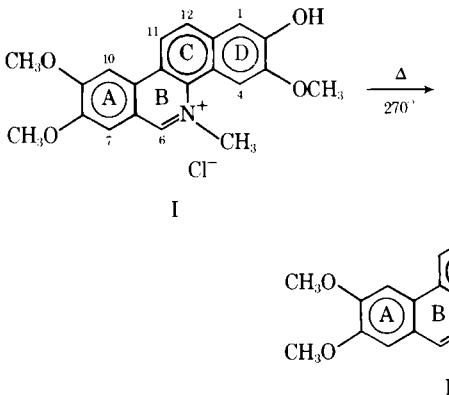
Keyphrases □ Fagaronine—structure confirmation □ Anticancer agents, potential—confirmation of fagaronine structure □ *Fagara zanthoxyloides* (Rutaceae) alkaloids—confirmation of fagaronine structure

Fagaronine (I), an alkaloid of *Fagara zanthoxyloides* (Rutaceae) (1), exhibits potent antileukemic properties and is currently being considered for preclinical evaluation by the National Cancer Institute. In an initial report, two possible structures for fagaronine were suggested, one of which was favored on the basis of mass spectral evidence. At that time, it was not possible to study the NMR spectrum in detail due to the poor solubility of I in all of the usual solvents. This problem has now been overcome by the preparation of *N*-demethylfagaronine (II) (Scheme I) which was found to be soluble in $CDCl_3$ and dimethyl sulfoxide-d_6 and from which good NMR data could be obtained.

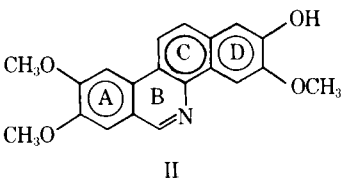
DISCUSSION

The NMR spectrum (Table I) of *N*-demethylfagaronine in $CDCl_3$ and that of its hydrogen-bonded complex with triethylamine showed a significant increase in shielding of an upfield aromatic proton (δ7.47) by 0.17 ppm and little or no effect on the other aromatic protons. This increase in shielding would be expected for protons *ortho* and *para* to the phenolic OH position (2). The proton at C-4, which could be expected to produce a downfield signal due to its orientation with respect to the ring B nitrogen and the accompanying electron pair, was not affected, which suggested that the phenolic group was located at the C-2 position.

The use of dimethyl sulfoxide-d_6 as the NMR solvent offered the advantage of determining in the same solvent the chemical shifts of the free *N*-demethyl base as well as those of the phenolate anion of the base. From the chemical shift changes, it was possible



I



II

Scheme I—Preparation of N-Demethylfagaronine from Fagaronine

Table I—NMR Data for *N*-Demethylfagaronine[a,b]

Protons	Chemical Shift (δ) in $CDCl_3$	Change (ppm) after Addition of Triethylamine[c]	Chemical Shift (δ) in Dimethyl Sulfoxide-d_6	Change (ppm) after Addition of NaOD
H_6	9.32	0	9.35	−0.08
H_4	8.82	0	8.68	−0.27
H_{11}	8.33 d, J = 9.0 Hz	−0.03	8.60 d, J = 9.0 Hz	−0.08
H_7 or H_{10}	7.93	0	8.17	−0.08
H_{12}	7.88 d, J = 9.0 Hz	−0.05	7.90 d, J = 9.0 Hz	−0.28
H_7 or H_{10}	7.43	0	7.72	−0.05
H_1	7.47	−0.17	7.37	−0.63
OCH_3	4.18	0	4.10 (A ring)	0
OCH_3	4.17	0	4.05 (C-3)	−0.10
OCH_3	4.07	0	3.98 (A ring)	0

[a] Chemical shifts measured downfield from internal tetramethylsilane. [b] Negative values indicate increased shielding. [c] Approximately 20% triethylamine.

not only to confirm the position of the phenolic group at C-2 but also to assign the remaining signals to the other aromatic protons. While the preparation of the phenolate by the addition of NaOD increased the shielding of all protons in the C and D rings due to the increased electron densities in these rings, it left the protons in the A and B rings largely unaffected.

The B ring proton singlet (H_6) was readily assigned by its most downfield position (δ9.35), which was due to its proximity to the ring nitrogen and by its insensitivity to anion formation. Two other singlets at δ7.72 and 8.17, unaffected by anion formation, were assigned to the remaining protons in the A ring, H_7 and H_{10}, and were not further differentiated from the data obtained.

In the study of Highet and Highet (2) of the chemical shift changes observed for 10 methyl- and methoxyphenols in dimethyl sulfoxide-d_6 upon anion formation, the magnitude of the shift changes offered a clear distinction between protons *ortho, meta,* and *para* to the OH site. They observed increased shielding for these protons at 0.50 ± 0.1, 0.28 ± 0.10, and 0.74 ± 0.05 ppm for the *ortho, meta,* and *para* protons, respectively.

The remaining aromatic singlets at δ7.37 and 8.68 in the spectrum of *N*-demethylfagaronine due to the D-ring protons gave shifts of 0.63 and 0.27 ppm, respectively, corresponding closely to those expected for *ortho* and *meta* protons. The only phenolic site possible consistent with these observations is at C-2 with the δ7.37 signal assigned to the H_1 proton *(ortho)* and the δ8.68 signal assigned to the H_4 proton *(meta)*. The downfield position predicted for the H_4 proton offered further verification of this assignment.

The two protons in the C ring constituted an *AB* system (J= 9.0 Hz) (Table I) and were both affected by the conversion of the phenolic group in the D ring to the phenolate, but by different magnitudes. The increased shielding shown by the upfield doublet (−0.28 *versus* −0.08 ppm) allows it to be assigned to the H_{12} proton by a consideration of the sites of increased electron density due to a phenolate group at C-2.

The methoxy group singlet at C-3 (δ4.05) could be distinguished readily from the signals due to the methoxy groups on the A ring since it alone shifted with anion formation.

From the data obtained on *N*-demethylfagaronine, the structure of fagaronine is thus confirmed as I.

EXPERIMENTAL[1]

Fagaronine was obtained as previously reported (1) from the roots of *Fagara zanthoxyloides* (Rutaceae). Fagaronine (100 mg) was heated at 270° for 5 min, during which time the original yellow color turned to a grayish white. The resulting product was mixed with 15 ml of chloroform, in which fagaronine is insoluble, and filtered. Following *in vacuo* removal of the chloroform, a 70-mg residue was obtained, which was purified by silica gel PF_{254} column chromatography, using an eluent of chloroform–methanol (9:1). The pure (TLC) *N*-demethylfagaronine (31 mg) exhibited a melting point of 250° and showed UV absorptions at λ_{max} 227 (log ϵ 4.30), 272 (4.67), and 280 (4.67) nm and a shoulder at 315 (4.04) nm. The IR spectrum was very similar to, but not identical with, that of fagaronine. The phenolic group was observed as a broad band at 3520 cm^{-1}. A molecular ion (M^+) was observed at *m/e* 335, followed by peaks at *m/e* 334, 320, 306, and 292 in the mass spectrum, the latter appearing very similar to the fragmentation pattern of fagaronine (1).

REFERENCES

(1) W. M. Messmer, M. Tin-Wa, H. H. S. Fong, C. Bevelle, N. R. Farnsworth, D. J. Abraham, and J. Trojánek, *J. Pharm. Sci.*, **61**, 1858(1972).

(2) R. J. Highet and P. F. Highet, *J. Org. Chem.*, **30**, 902(1965).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 18, 1974, from the *Department of Pharmacognosy and Pharmacology and the Department of Medicinal Chemistry, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612*

Accepted for publication April 24, 1974.

Supported by Research Contract CM-22078 from the National Cancer Institute, Bethesda, MD 20014

[x] To whom inquiries should be directed.

[1] All NMR spectra were determined using a Varian T-60A instrument.