# PLANT ANTITUMOR AGENTS, 27.[1] ISOLATION, STRUCTURE, AND STRUCTURE ACTIVITY RELATIONSHIPS OF ALKALOIDS FROM *FAGARA MACROPHYLLA*

MONROE E. WALL,* M.C. WANI,* and HAROLD TAYLOR

Research Triangle Institute, P.O. Box 12194, Research Triangle Park, North Carolina 27709

ABSTRACT.—The known alkaloids nitidine chloride [1] and 6-oxynitidine [2] and a new compound 6-methoxy-5,6-dihydronitidine [3] have been isolated from *Fagara macrophylla*. Compound 3 was the major product and was shown to be an artifact. The alkaloids 1 and 3 have been interconverted by treatment of 1 under basic conditions or 3 under acidic conditions. On sublimation 1 and 3 formed 8,9-dimethoxy-2,3-methylenedioxybenzo[c]phenanthridine [4] which could then be converted to 5,6-dihydronitidine [5]. The alkaloids 1 and 3 are about equipotent in P-388 mouse leukemia, giving high T/C values of 240-260% at doses of 30-50 mg/kg. The other compounds were inactive. The structural requirement for antitumor activity in the phenanthridine series is the ability to form a C-6 iminium ion.

Fagara macrophylla Engl. is a member of the Rutaceae family. Many *Fagara* and related *Zanthoxylum* species have been used in folk medicine as remedies for a variety of ailments (1). In 1971, we presented a preliminary report that, for the first time, showed that several alkaloids isolated from extracts of the bark of *F. macrophylla* had potent activity against P-388 mouse leukemia (2). These compounds were the known benzo[c]phenanthridine alkaloids nitidine chloride [1], 6-oxynitidine [2], and a new compound, 6-methoxy-5,6-dihydronitidine [3]. Compounds 1 and 2 were first described by Arthur and Ng (3). In this paper we wish to present the full experimental de-

1

2

3

4

5
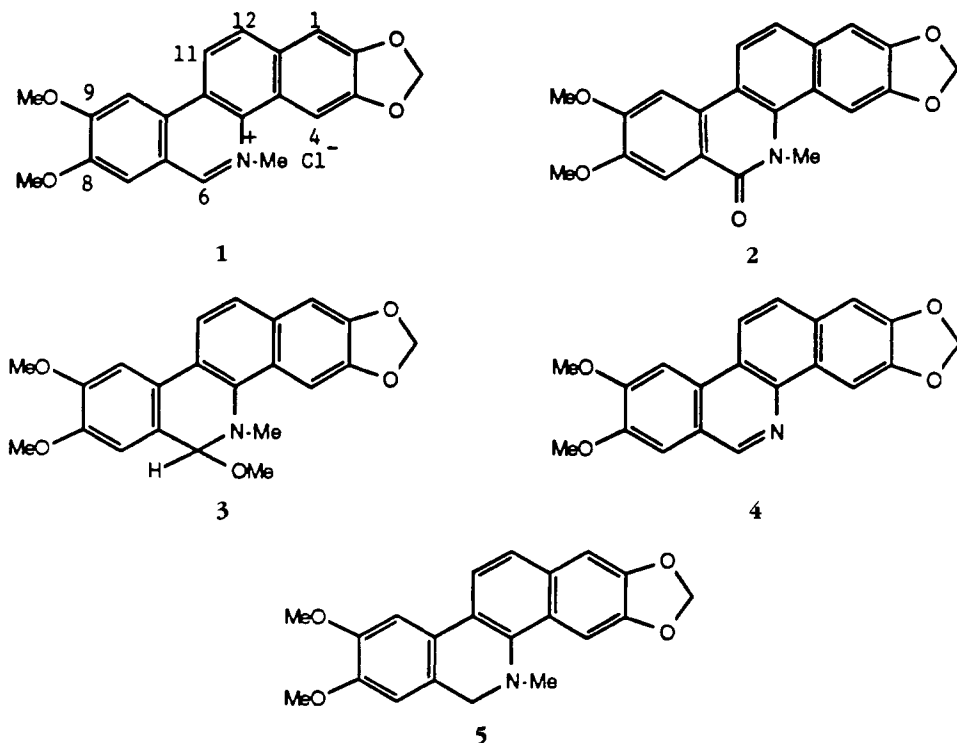
[1]For Part 26 in this series, see M.E. Wall, M.C. Wani, B.N. Meyer, and H.L. Taylor, "Isolation, Structure and Antitumor Activity of Alkaloids from *Anopterus glandulosus," J. Nat. Prod.,* **50**, 1152 (1987).

tails of our investigations with emphasis on the antitumor activity and SAR relation-
ships of the various alkaloids. In addition, we wish to present full information on the
physical properties (hrms, ir, and ^{1}H nmr) of these compounds and chromatographic
data, all of which are absent in the original report (3).

RESULTS AND DISCUSSION

Antitumor testing of the crude EtOH extracts of the bark of *F. macrophylla* in the
9KB in vitro cytotoxicity and the P-388 in vivo mouse leukemia systems showed that
the crude extracts had confirmed activity of a high order in these assays (4). After hot
EtOH extraction of a 9-kg sample of bark followed by partial concentration, large
quantities (see Experimental section) of a precipitate FO28 were obtained, which were
highly active in the P-388 leukemia system. Extraction of the latter with Me$_2$CO gave
an Me$_2$CO-insoluble fraction that retained biological activity. After dissolution of this
fraction in 4% NH$_4$OH, extraction with CHCl$_3$, concentration, and heating in
MeOH/CHCl$_3$, a crystalline product F1 was obtained. The mother liquors were
chromatographed on Sephadex LH-20, and a small crystalline fraction F2 and a much
larger crystalline fraction F3 were obtained. F1 and F3 were identical and were shown to
be 6-methoxy-5,6-dihydronitidine [3], and F2 was shown to be nitidine chloride [1].
As will be shown below, 3 is undoubtedly an artifact formed from 1 by the method of
isolation. 6-Oxynitidine [2] was obtained in low yield from a crude alkaloid fraction
obtained from the EtOH-soluble fraction after filtration of the precipitate FO28 de-
scribed above. Compounds 1, 2, and 3 were further characterized by hrms, ir, and ^{1}H
nmr (see Experimental section).

The ir spectra of all three compounds showed the presence of aromatic C=C and
methylenedioxy and methoxy absorptions around 1600 and 900-1100 cm^{-1}, respec-
tively. In addition, the ir spectrum of 2 showed the absorption due to the six-mem-
bered ring lactam at 1640 cm^{-1}. Based on literature precedence (5), the characteristic
signals due to the N-CH$_3$, OMe, OCH$_2$O, and aromatic protons in the ^{1}H-nmr spectra
of 1, 2, and 3 could be readily assigned. In the spectra of 1 and 3 the singlet due to 6-H
appeared at δ 9.88 and 5.06, respectively, and this signal was absent in the spectrum of
2.

STRUCTURE INTERRELATIONSHIPS.—Alkaloids 1 and 3 are readily interconvert-
ible. Treatment of 3 with methanolic HCl gives a quantitative yield of 1; the latter on
treatment with MeOH containing NH$_3$ gives 3. Since MeOH/CHCl$_3$ was frequently
used in the isolation described above, there is little doubt that 3 is at least to some ex-
tent an artifact. Further confirmation that 3 is an artifact is based on similar findings for
the closely related 7,8-dimethoxy alkaloid chelerythrine from which 6-ethoxy- and 6-
methoxy-chelerythrine were formed after basification and crystallization from EtOH or
MeOH, respectively (6,7). Isolation of 1 as the quaternary chloride may also not reflect
the exact nature of the anion in the plant. Sublimation of 1 and 3 at 200°/0.005 mm,
converted both to the same compound 4 that was synthesized by Arthur and Ng (8) and
can be regarded as the parent benzophenanthridine in this series, i.e., 8,9-dimethoxy-
2,3-methylenedioxybenzo[c]phenanthridine. We have further characterized 4 by
hrms, ir, and ^{1}H nmr. The ir spectrum of 4 showed characteristic methylenedioxy ab-
sorption bands at 1035 cm^{-1} and C=N band at 1620 cm^{-1}. The ^{1}H-nmr spectrum
showed the singlet due to 6-H at δ 9.2. Compound 4 was converted to the methosulfate
and reduced by Zn-HCl to give the known (8) 5,6-dihydronitidine [5] which was
further characterized by hrms, ir, and ^{1}H nmr. The ir spectrum of 5 was characterized
by the absence of C=N absorption and its ^{1}H-nmr spectrum showed the 6-H as a
singlet at δ 4.15.

ANTITUMOR ACTIVITY.—Compounds **1** and **3** had comparable activity. Both exhibited 9KB cytotoxicity on the order of $ED_{50} = 1 \times 10^{-1}$-$2 \times 10^{-1}$. Both were highly active in P-388 mouse leukemia, giving comparable maximal T/C values as high as 240-260% at doses of 30-50 mg/kg by standard ip injection of compounds (4). Considering the fact that **1** and **3** are mutually interconvertible, it is not improbable that each would give an equilibrium mixture of **1** $\rightleftharpoons$ **3** at physiological pH. Giving further credence to this belief are the results of a recent study of the effects of pH on the iminium ion concentration in sanguinarine, a 7,8-methylenedioxy member of the benzo[*c*]phenanthridine alkaloids (9). In this study iminium ion fell from 88% at pH 6.0 to 41% at 6.6 and to 5% at 7.0. Both **1** and **3** failed to show high enough activity in other antitumor assays to be considered for clinical trial. Thus, both **1** and **3** were only marginally active in L-1210 mouse leukemia and were inactive in B16 melanoma and Lewisburg life-prolongation assay. A closely related alkaloid, fagaronine, which differs from **1** only in that the 2,3-methylenedioxy moiety in **1** is replaced by a 2-hydroxy-3-methoxy functionality, was isolated by Farnsworth *et al.* (10,11) shortly after our initial report. The activity of fagaronine in P-388 was very high (of the same order as **1** and **3**). However, this compound also has not had sufficient activity in other test systems to receive further study.

An excellent review of the chemistry and biological activity of all the benzo[*c*]phenanthridine alkaloids has been presented recently by Suffness and Cordell (12). It is apparent that the nature of 2,3 substituents is not critical for the P-388 activity. What is critical is the location of the dimethoxy groups in ring A. Stermitz *et al.* prepared by total synthesis a number of analogs of sanguinarine and chelerythrine. In the latter, a compound identical with nitidine chloride [**1**] was prepared except for the presence of a 7,8-dimethoxy moiety rather than the 8,9-dimethoxy group of the latter (13). This compound was inactive in P-388. 6-Oxynitidine [**2**] was inactive in P-388 and less active in 9KB than **1** or **3**. The other compounds **4** and **5** were likewise inactive in P-388. It would seem that the important site of the molecule is in the $C=N^+$ region because the carbon of the iminium function represents an effective alkylation site (12). Blocking this region by the steric hindrance of the peri-group at C-7 in chelerythrine derivatives, or removing the iminum function by formation of the 6-oxo group in **2**, or by conversion to 5,6-dihydronitidine [**5**] leads to inactivation of the alkaloid.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot-stage microscope and are uncorrected. ^{1}H-nmr spectra were obtained with a Varian A100 or with a Bruker WM-250 spectrometer using TMS as internal standard. High resolution mass spectra were obtained with an AEI MS-902 or on a VG ZAB E mass spectrometer using fast atom bombardment ionization instrument. Ir spectra were measured as KBr discs or $CHCl_3$ solutions with a Perkin-Elmer 267 spectrophotometer. Uv spectra were measured in MeOH using a Cary-14 instrument. Preparative chromatography was conducted on Sephadex LH 20 columns.

EXTRACTION AND FRACTIONATION.—Plant material, stem/bark of *F. macrophylla* (PR-15195; 9.1 kg) was collected in Ghana in 1967.[2] It was extracted exhaustively at 50° with 95% EtOH in a continuous, pilot plant scale extractor (continuous, hot percolation type) for 48 h with several solvent changes. The EtOH extracts were combined and concentrated to a volume of 4 liters. Upon cooling, a precipitate was formed that was collected by filtration. This precipitate, FO28, weighed ca. 85 g, and was highly active in P-388. The filtrate was concentrated in vacuo to remove most of the remaining EtOH; the residue

was triturated with 4 liters of 5% aqueous HCl at ca. 50°. The HCl-soluble fraction was decanted; the insoluble fraction FO29 weighed 45 g and was much less active than FO28. The HCl-soluble fraction was extracted four times with 1.5 liters of CHCl₃ each time. The CHCl₃-soluble fraction FO30 weighed 205 g and was inactive in P-388. The CHCl₃-insoluble aqueous fraction was basified with NH₃ and again extracted with CHCl₃. This fraction, FO31, weighed 9.5 g and was highly active in P-388. The remaining material was discarded.

ISOLATION OF NITIDINE CHLORIDE [1] AND 6-METHOXY-5,6-DIHYDRONITIDINE [3].—An aliquot (41.0 g) of FO28 was continuously extracted overnight with Me₂CO in a Soxhlet extractor. The Me₂CO-insoluble residue was disolved in 4% NH₄OH and partitioned with CHCl₃. The CHCl₃ fraction weighed 9.8 g. The majority of this fraction was dissolved in 40 mL of MeOH-CHCL₃ (3:1) and allowed to stand. A crystalline product F1 (1.4 g) deposited, which was shown to be 6-methoxy-5,6-dihydronitidine [3]. The mother liquor (8.0 g) was chromatographed on Sephadex LH-20 (1300 g) in MeOH-CHCl₃ (3:1). A crystalline product F2 (tubes 49-52) weighing 0.05 g was shown to be nitidine chloride [1]. The other crystalline product F3 (tubes 74-86) weighing 1.86 g was also shown to be 6-methoxy-5,6-dihydronitidine [3].

PHYSICAL CONSTANTS OF NITIDINE CHLORIDE [1].—This compound, on being heated to about 240°, changes to a colorless liquid that melts at 281-282° [lit. (3) 285-286°]; uv (MeOH) 232 (4.41), 272 (4.48), 329 (4.38); ir (KBr) 1600 (aromatic C=C), 1030 (OCH₂O) cm⁻¹; ¹H nmr (DMSO-d_6) 250 MHz δ 4.05 (s, 3, OCH₃), 4.23 (s, 3, OCH₃), 4.90 (s, 3, NCH₃), 6.35 (s, 2, OCH₂O), 7.77 (s, 1, ArH), 7.90 (s, 1, ArH), 8.27 (d, 1, J=9 Hz, 12-H), 8.31 (s, 1, ArH), 8.36 (s, 1, ArH), 8.89 (d, 1, J=9 Hz, 11-H), 9.88 (s, 1, 6-H); hrfabms m/z 383.0923 (C₂₁H₁₈NO₄Cl requires 383.0925).

Anal. calcd for C₂₁H₁₈NO₄Cl·2.5H₂O: C, 58.82; H, 5.40; N, 3.26; Cl, 8.26. Found: C, 58.98; H, 4.62; N, 3.41; Cl, 8.50.

PHYSICAL CONSTANTS OF 6-METHOXY-5,6-DIHYDRONITIDINE [3].—This compound changes its crystalline form between 180° and 200° followed by gradual softening and final melting at 270°; uv (MeOH) 235 (4.29), 270 (4.54), 292 (4.51), 328 (4.41); ir (KBr) 1600 (aromatic C=C), 1035 (OCH₂O) cm⁻¹; ¹H nmr (CDCl₃) 100 MHz δ 2.76 (s, 3, NCH₃), 3.44 (s, 3, 6-OCH₃), 3.96 (s, 3, OCH₃), 4.00 (s, 3, OCH₃), 5.06 (s, 1, 6-H), 6.02 (s, 2, OCH₂O), 6.94 (s, 1, ArH), 7.16 (s, 1, 1-H), 7.38 (s, 1, ArH), 7.46 (d, 1, J=9 Hz, 12-H), 7.66 (s, 1, 4-H), 7.74 (d, 1, J=9 Hz, 11-H); hrms m/z 379.1412 (requires C₂₂H₂₁NO₅ 379.1419).

Anal. calcd for C₂₂H₂₁NO₅: C, 69.64; H, 5.58; N, 3.69. Found: C, 69.76; H, 5.63; N, 3.68.

ISOLATION OF 6-OXYNITIDINE [2].—Fraction FO31 (see above) was chromatographed on Sephadex LH-20 (1400 g) in MeOH. Fraction FO46 (tubes 305-355) weighing 0.77 g was highly active in 9KB assay and gave a positive Dragendorff test. This fraction was successively triturated with C₆H₆ and MeOH, and the insoluble material was crystallized from MeOH-CHCl₃ (1:1) to give 6-oxynitidine [2] (76 mg), mp 282-283° [lit. (3) 284-285°]; ir (KBr) 1640 (six-membered lactam), 1610 (aromatic C=C), 1035 (OCH₂O) cm⁻¹; ¹H nmr (CDCl₃) 100 mHz δ 3.95 (s, 3, NCH₃), 4.04 (s, 3, OCH₃), 4.08 (s, 3, OCH₃), 6.09 (s, 2, OCH₂O), 7.13 (s, 1, 1-H), 7.45 (d, 1, J=9 Hz, ArH), 7.54 (s, 1, 4-H or 10-H), 7.61 (s, 1, ArH), 7.92 (s, 1, ArH, 7-H), 7.95 (d, 1, J=9 Hz, 11-H); hrms m/z 363.1098 (C₂₁H₁₇NO₅ requires 363.1107).

6-METHOXY-5,6-DIHYDRONITIDINE [3] FROM NITIDINE CHLORIDE [1].—A suspension of 1 g of 1 in 160 ml of 28.4% NH₄OH was stirred at room temperature for 0.5 h and then extracted with 600 ml of EtOAc. Evaporation of the dry EtOAc extract gave a syrupy material which was dissolved in 60 ml of hot MeOH. Concentration of the methanolic solution afforded 0.720 g (81%) of crystalline 3 identical with the product isolated from natural plant material.

8,9-DIMETHOXY-2,3-METHYLENEDIOXYBENZO[c]PHENANTHRIDINE [4].—*From nitidine choride* [1].—Sublimation (220°/0.005 mm) of 1 (30 mg) gave 4 (22 mg).

From 6-methoxy-5,6-dihydronitidine [3].—Sublimation of 3 (150 mg) under similar conditions also gave 4 (35 mg): 274-280° [lit. (8): 273°]; uv (MeOH) 228 (4.42), 273 (4.77), 280 (4.76), 314 (4.24); ir (KBr) 1625 (C=N), 1600 (aromatic C=C), 1035 (OCH₂O) cm⁻¹; ¹H nmr (CDCl₃) 100 MHz δ 4.06 (s, 3, OCH₃), 4.11 (s, 3, OCH₃), 6.12 (s, 2, OCH₂O), 7.36 (s, 1, ArH), 7.80 (d, 1, J=9 Hz, 12-H), 7.86 (s, 1, ArH), 8.28 (d, 1, J=9 Hz, 11-H), 8.68 (s, 1, ArH), 9.20 (s, 1, 6-H); hrms m/z 333.1005 (C₂₀H₁₅NO₄ requires 333.1001).

Anal. calcd for C₂₀H₁₅NO₄: C, 72.06; H, 4.54; N, 4.20. Found: C, 71.98; H, 4.57; N, 4.16.

5,6-DIHYDRONITIDINE [5].—The title compound was prepared from 4 by a known literature procedure (7) for further characterization and biological evaluation. Thus, a solution of 4 (31.6 mg) in xylene (0.3 ml) and nitrobenzene (0.6 ml) containing dimethyl sulfate (0.06 ml) was refluxed for 5 min. The reac-

tion mixture was cooled, and the precipitated methosulfate was removed by filtration and washed with Et_2O.

A solution of the above methosulfate (42 mg) in H_2O (6 ml) and concentrated HCl (0.4 ml) containing zinc dust (0.8 g) was refluxed under nitrogen for 5 h, more acid (0.3 ml) being added every hour. The cooled reaction mixture was made basic with NH_3 and extracted with $CHCl_3$. The residue from the dried (Na_2SO_4) $CHCl_3$ extract was purified by elution from SiO_2 (5 g) using 2% Me_2CO in $CHCl_3$ to give **5** (14 mg), mp 208-210° [lit. (8): 208-211°]; uv (MeOH) 228 (4.63), 278 (4.56), 310 (4.32); ir (KBr) 1600 (aromatic C=C), 1035 (OCH_2O) cm^{-1}; ^{1}H nmr ($CDCl_3$) 100 MHz δ 2.62 (s, 3, NMe), 3.94 (s, 3, OMe), 3.98 (s, 3, OMe), 4.15 (s, 2, 6-H), 6.02 (s, 2, OCH_2O), 6.79 (s, 1, ArH), 7.08 (s, 1, 1-H), 7.30 (s, 1, ArH), 7.45 (d, 1, J=9 Hz, 12-H), 7.65 (s, 1, 4-H), 7.70 (d, 1, J=9 Hz, 11-H); hrms m/z 349.1310 ($C_{21}H_{19}NO_4$ requires 349.1314).

ACKNOWLEDGMENTS

LITERATURE CITED

1.   W.H. Lewis and M.P.F. Elvin-Lewis, "Medical Botany," John Wiley and Sons, New York, 1977, pp. 237-238.
2.   M.E. Wall, M.C. Wani, and H.L. Taylor, Abstracts of Papers 162nd Meeting, American Chemical Society, Washington, DC, 1971; MEDI p. 34.
3.   H.R. Arthur, W.H. Hui, and Y.L. Ng, *J. Chem. Soc.*, 1840 (1959).
4.   R.I. Geran, N.H. Greenberg, M.M. Macdonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep.*, **3**, 1 (1972).
5.   M. Onda, K. Abe, K. Yonezawa, N. Esumi, and T. Suzuki, *Chem. Pharm. Bull. (Japan)*, **18**, 1435 (1970).
6.   P.J. Scheuer, M.Y. Chang, and C.E. Swanson, *J. Org. Chem.*, **27**, 1472 (1962).
7.   D.B. MacLean, D.E.F. Gracey, J.K. Saunders, R. Rodrigo, and R.H.E. Manske, *Can. J. Chem.*, **47**, 1951 (1969).
8.   H.R. Arthur and Y.L. Ng, *J. Chem. Soc.*, 4010 (1959).
9.   R.R. Jones, R.J. Harkrader, and G.L. Southard, *J. Nat. Prod.*, **49**, 1109 (1986).
10. W.M. Messmer, M. Tin-Wa, H.S. Fong, C. Benelle, and N.R. Farnsworth, *J. Pharm. Sci.*, **61**, 1858 (1972).
11. M. Tin-Wa, C.L. Bell, C. Benelle, H.S. Fong, and N.R. Farnsworth, *J. Pharm. Sci.*, **63**, 1476 (1979).
12. M. Suffness and G.A. Cordell, in: "The Alkaloids," Ed. by A. Brossi, **25**, Academic Press, New York, 1985, pp. 178-188.
13. F.R. Stermitz, K.A. Larson, and D.K. Kim, *J. Med. Chem.*, **16**, 939 (1973).