Journal of Natural Products
Vol. 50, No. 5, pp. 891-896, Sep-Oct 1987

CONSTITUENTS OF *PARAMICHELIA BAILLONII*: A NEW ANTITUMOR GERMACRANOLIDE ALKALOID[1]

NIJSIRI RUANGRUNGSI, ARTHORN RIVEPIBOON,

Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10500, Thailand

GORDON L. LANGE,* MOSES LEE, CARL P. DECICCO,

Guelph-Waterloo Centre for Graduate Work in Chemistry, Department of Chemistry and Biochemistry, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

PORNTIPA PICHA, and KANLAYA PREECHANUKOOL

Research Division, National Cancer Institute, Bangkok 10400, Thailand

ABSTRACT.—*Paramichelia baillonii* has been used by the natives of Northern Thailand for medicinal purposes. Four components have been isolated from the bark of this plant and their structures determined by spectroscopic means. Three of the components had been reported previously: the germacranolide epoxides (−)-dihydroparthenolide [**1**] and (−)-parthenolide [**2**] and the oxoaporphinoid alkaloid liriodenine [**4**]. The fourth component is an unusual new germacranolide alkaloid which has been named (−)-bisparthenolidine [**3**] because it was presumably formed in the plant from ammonia and two molecules of parthenolide. Parthenolide was reported previously to possess anti-tumor activity, and in the present study we report on the significant activity of the new alkaloid **3** in the KB cell culture assay.

The genus *Paramichelia* Hu in the family Magnoliaceae has three species distributed in south and southeast Asia (2). This genus is very similar to that of *Michelia* L. in both external features and ethnomedical properties. *Paramichelia baillonii* (Pierre) Hu [synonymous with *Magnolia baillonii* Pierre, *Michelia baillonii* (Pierre) Fin. and Gagnep., *Aromadendron spongocarpum* (King) Craib., and *A. baillonii* (Pierre) Craib.] is the only species found in northern Thailand and is known as "champi pa" (3,4). The bitter bark of this plant has been used by the natives as a stimulant, febrifuge (5), and as a substitute for champaca bark (*Michelia champaca* L.). A decoction of the bark of *M. champaca* has been used as a febrifuge, as a protective medicine for mothers after childbirth (5,6), and in India for the treatment of abdominal tumors (7). There have been no previous reports of phytochemical studies on any part of *P. baillonii*, and in this article we report the structural elucidation of four constituents of the bark of this plant.

Chromatographic purification of the alcoholic extract of *P. baillonii*, as described in the Experimental section, provided the four components to be discussed. Examination of the spectroscopic data and the optical rotation of **1** established clearly that this least polar component was (−)-dihydroparthenolide. The 400 MHz ^{1}H-nmr spectrum of **1** is reported in Table 1 as previously only low resolution spectra with few proton assignments reported (8,9). The ^{13}C spectrum of **1** is reported for the first time in Table 2. (−)-Dihydroparthenolide has been isolated previously from *Michelia lanuginosa* (10), *Michelia compressa* (9), and *Ambrosia artemisiifolia* L. (11). Similarly, component **2** was shown to be (−)-parthenolide on the basis of spectroscopic comparisons with literature data. The 200 MHz ^{1}H assignments (12) for **2** are included in Table 1 for comparison with **1** and **3** and in a footnote we report additional assignments obtained from our 400 MHz spectrum and an ^{1}H-^{1}H 2D-COSY experiment. The previously reported ^{13}C spectrum of **2** (13) is included in Table 2 for comparison with the spectra of **1** and **3**, also. The structure and conformation of **2** were previously established unambiguously

[1]Part IV in the series of "Studies on Thai Medicinal Plants." For Part III, see Ruangrungsi *et al.* (1).

TABLE 1. ^{1}H-nmr Spectra of **1**, **2**, and **3**[a]

Proton	Compounds		
	1	**2**[b]	**3**
1	5.15 (dd, 2.3, 11.9)	5.21 (dd, br, 4.0, 12.2)	5.27 (dd, 2.2, 9.8)
2α	2.11 (dddd, 2.3, 6.0, 13.0, 13.0)	2.09-2.24 (m)[c]	2.26 (dd, 6.0, 12.1)
2β	2.37 (dddd, 5.0, 11.9, 13.3, 13.0)	2.46 (ddd, 13.8, 12.2, 12.5)	2.40 (m)
3α	1.21 (ddd, 6.0, 13.0, 13.0)	1.25 (m)	1.23 (dt, 5.9, 13.9)
3β	2.16 (m)	2.09-2.24 (m)[c]	1.88 (dd, 5.9, 14.6)
5	2.69 (d, 9.0)	2.79 (d, 8.9)	2.74 (d, 8.8)
6	3.80 (dd, 8.4, 9.0)	3.86 (dd, 8.9, 8.3)	3.86 (t, 8.8)
7	2.28 (m)	2.78 (m)	2.40 (m)
8α	2.28 (m)	2.09-2.24 (m)[c]	2.18 (m)
8β	1.80 (m)	1.73 (m)	1.70 (m)
9α	1.80 (m)	2.09-2.24 (m)	2.10-2.18
9β	2.25 (m)	2.38 (m)	
11β	2.27 (dq, 6.8, 10.3)	—	2.40 (m)
13a	1.25 (d, 6.8, CH_3)[d]	6.34 (d, 3.6)	3.15 (dd, 2.8, 13.1)
13b		5.62 (d, 3.1)	2.92 (dd, 2.8, 13.1)
14	1.68 (s)	1.72 (s)	1.67 (s)
15	1.27 (s)[d]	1.31 (s)	1.30 (s)

[a]Chemical shifts are in ppm from TMS, coupling constants are in parentheses in Hertz, and the samples, were dissolved in $CDCl_3$.

[b]Previously assigned 200 MHz spectrum from Badesinsky *et al.* (12).

[c]Specific assignments possible at 400 MHz with 2D-COSY and decoupling experiments; $H_{2\alpha}$, 2.38 (dd, 5.1, 13.1); $H_{3\beta}$, 2.17 (m); $H_{8\alpha}$, 1.72 (m).

[d]In C_6D_6 solvent the C-13 and C-15 methyls were clearly resolved into a doublet at δ 1.05 and a singlet at 0.98, respectively.

TABLE 2. ^{13}C-nmr Spectra of **1**, **2**, and **3**[a]

Carbon	Compounds		
	1	**2**[b]	**3**
1	125.1 (−)	125.3	125.3 (−)
2	24.0 (+)[c]	24.2[c]	24.2 (+)[c]
3	36.6 (+)[c]	36.2[c]	36.5 (+)[c]
4	61.4 (+)	61.5	61.6 (+)
5	66.3 (−)	66.4	66.1 (−)
6	82.1 (−)	82.5	82.3 (−)
7	51.9 (−)	47.7	49.0 (−)
8	29.7 (+)[c]	41.2[c]	30.2 (+)[c]
9	41.1 (+)	30.2[c,d]	40.9 (+)
10	134.4 (+)	134.7	134.3 (+)
11	42.4 (−)	139.5	45.5 (−)
12	179.6 (+)	169.3	176.7 (+)
13	13.2 (−)	121.0	46.2 (+)
14	17.1 (−)	17.3	17.2 (−)
15	16.8 (−)	17.0	16.8 (−)

[a]Chemical shifts are in ppm from TMS, solvent was $CDCl_3$, (+) and (−) are signs from the attached proton test.

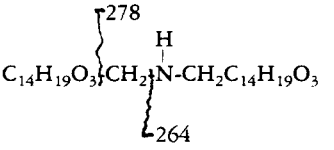
[b]Data taken from El-Feraly *et al.* (13).

[c]Assignments may be interchanged.

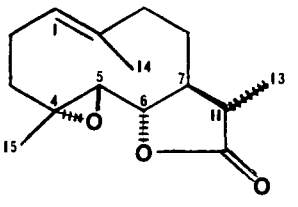
[d]We believe the 41.2 ppm resonance should be assigned to C-9.

by single crystal X-ray analysis (14), and the optical rotation of this component from *P. baillonii* showed that it possessed the (6*S*) absolute configuration as depicted in **2** (15).

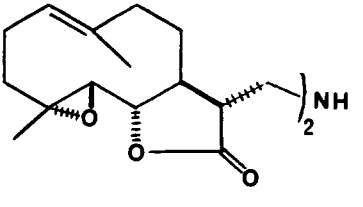
The third component, **3**, was a crystalline solid and its eims (parent peak *m/z* 513, base peak 278) was consistent with a compound containing two sesquiterpenoid units and one nitrogen atom. Accurate mass determinations of the parent peak and two fragments (*m/z* 278 and 264, see Experimental section) further supported the presence of a nitrogen atom and fragmentations as indicated below:



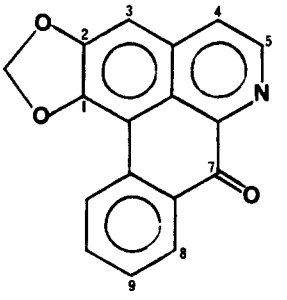
The ir spectrum displayed a strong absorption at 1770 (γ-lactone) and a weak band at 3365 cm^{-1} (N-H). Both the ^{1}H- and the ^{13}C-nmr spectra (Tables 1 and 2, respectively) showed many similarities to the spectra of dihydroparthenolide [**1**] with the only significant differences being in the region of C(13). In particular, the two protons on C(13) in **3** appeared as an AB pattern (J=13.1 Hz) at about 3 ppm with additional splitting (J=2.8 Hz), and in the ^{13}C spectrum the chemical shift of C(13) (46.2 ppm) and the attached proton test (APT) were consistent with the presence of a nitrogen atom on a methylene carbon. Assignments for the protons of **3** given in Table 1 were aided by a 2D-COSY experiment. On the basis of this spectroscopic information, we propose that this component is the sesquiterpenoid alkaloid **3**, formed by Michael addition of ammonia to two molecules of parthenolide. The aminomethyl group at C(11) is tentatively assigned the α-configuration because in the ^{1}H-nmr spectrum of **3** in C_6H_6 solvent, H(7) appears as a well-resolved quartet (J=8.8 Hz) as a result of *trans* couplings with H(6), H(8β), and H(11). Presumably **3** is derived from (−)-parthenolide, which has a (6*S*)-configuration (15), so the same absolute configuration is assigned to this new alkaloid, which we have chosen to call bisparthenolidine.



1
2=11,13-dehydro **1**

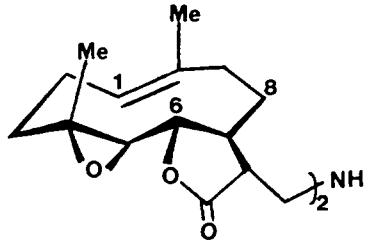


3



4

As component **3** is a new germacranolide derivative, we were interested in determining its conformation in solution using ^{1}H-nOe experiments. Low-intensity irradiation of a degassed $CDCl_3$ solution of **3** at 1.67 ppm [the resonance for the C(14) methyl group] caused an increase in the intensity of the signals for H(2β), H(8β), and H(9β) of 43, 43, and 10%, respectively. In addition, irradiation of the H(6) resonance at 3.86 ppm resulted in a 47 and 9% enhancement of the H(8β) and C(15) methyl signals, respectively. Because there was interaction between the C(15) methyl group and H(6) but none between H(5) and H(6), the *trans* configuration of the C(4)-C(5) epoxide was confirmed. A 2D-nOe experiment (NOESY) (16) confirmed the interactions mentioned above but also revealed a weak cross-peak correlation between the C(14) and C(15) methyl signals, thus indicating the *syn* relationship between these two groups. The nOe results clearly indicate a conformation for this parthenolide derivative as shown in **3a**. A similar conformation has been reported for parthenolide [**2**] (14).



3a

As component **3** is a new and unusual natural product it deserves some comment. At no point in the isolation procedure was NH_3 used, and, thus, we are confident that **3** is not an artifact. The ^{13}C-nmr spectrum of **3** shows only fifteen lines as would be expected for one pure diastereomer; a mixture of diastereomers would be expected if the component was formed chemically rather than in the plant. To our knowledge, **3** is the first reported example of a naturally-occurring germacranolide alkaloid, although a piperidine adduct of a pseudoguaianolide (17) and a tertiary amine derived from NH_3 and three molecules of α-methylenebutyrolactone (18) have been isolated previously from natural sources. A secondary amine related to **3** has been synthesized from NH_3 and two molecules of the eudesmanolide, alantolactone (19), and there have been numerous reports of reactions of secondary amines with the α-methylene group of germacranolides (20-21).

The fourth and most polar component isolated from *P. baillonii* was a high-melting, yellow, crystalline solid. Comparison of its ei mass spectrum (22), ir (23,24) and uv spectra (24,25), ^{1}H- (9,26) and ^{13}C-nmr spectra (27) with literature data established unambiguously that **4** was the alkaloid liriodenine. Liriodenine has some solubility in 10% DMSO-d_6/$CDCl_3$ and in the Experimental we report the 400 MHz ^{1}H-nmr spectra of **4** in this solvent as previous nmr reports only assigned some of the protons. To assign all the aromatic protons a 2D-COSY experiment was performed. It showed clearly that the doublet for H(11) at 8.72 was coupled to the triplet for H(10) at 7.77 and that this latter proton was also coupled with H(9) at 7.58 ppm. The remaining downfield doublet for H(8) at 8.57 ppm was also coupled with H(9). Liriodenine has previously been reported to be present in a number of different Magnoliaceae genera (28,29).

Because *P. baillonii* has been used by Thai natives for medicinal purposes, some of the biological activities of the components isolated are noted below. Parthenolide [**2**] demonstrates significant activity against the human laryngeal epidermoid carcinoma

(ED_{50}=0.76) (30) and the 9KB cell culture system (ED_{50}=0.45) (9); while dihydroparthenolide is inactive in the latter assay (9). Liriodenine [**4**] shows significant cytotoxicity against the 9KB system [ED_{50}=1.6 (31) or 3.8 (9)] and also exhibits a wide range of antimicrobial activity in vitro (32). In the present study, the cytotoxicity of the new alkaloid bisparthenolidine [**3**] was examined and was found to have a significant ED_{50} of 0.60 μg/ml in the KB cell culture assay. Normally Michael addition of a nucleophile to an α-methylene-γ-lactone moiety to give an adduct such as in **3** would be expected to reduce or destroy the biological activity of the compound (33), but the plant may be using this adduct as a storage mechanism and upon β-elimination of the amino group, the active component **2** is generated (33).

EXPERIMENTAL

INSTRUMENTATION.—^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker WH 400 spectrometer with TMS (δ=0) as internal standard and with solvents as indicated. Ir spectra were obtained on a Perkin-Elmer Model 1330 or 180 spectrometer, uv spectra on a Varian DMS 90 spectrophotometer, and mass spectra on a Varian MAT CH7 or VG Micromass 7070F spectrometer. Optical rotations were performed in a Bendix-NPL automatic polarimeter. The tlc analyses were performed on Si gel GF 264 plates of thickness 0.25 mm.

PLANT MATERIAL.—The bark of *P. baillonii* was collected from Doi Suthep, Chiang Mai Province, Thailand, in July 1985. Authentication was achieved by comparison with herbarium specimens at the Royal Forest Department, Ministry of Agriculture and Cooperatives, Thailand. A voucher specimen of plant material has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

EXTRACTION AND PURIFICATION.—The fresh bark of *P. baillonii* (3 kg) was blended with 95% EtOH, macerated twice over a period of 3 days (10 and 5 liters), and then filtered. The filtrate was concentrated under reduced pressure to give a residue which was treated with H_2O (5 liters), followed by extraction with $CHCl_3$ (3 times, 2 liters). The combined organic extracts were dried (anhydrous Na_2SO_4) and removal of the solvent gave a residue (8.59 g), which was chromatographed on a Si gel column (8×15 cm). The products were eluted with a 70% EtOAc/petrol solvent system, and 25 ml fractions were collected. Fractions 67-126 afforded a crude mixture (0.52 g), which was further purified using the following sequence of solvents and collecting 25-ml fractions: (a) 50% $CHCl_3$/petrol, 25 fractions, (b) $CHCl_3$, 10 fractions, and (c) $CHCl_3$-Me_2CO (8:2), 15 fractions. Fractions 11-12 gave 85 mg of **1**, fractions 28-36 gave 136 mg of **3**, and fractions 49-50 gave 97 mg of a mixture which is presently under investigation.

After increasing the polarity of the solvent in the large 8×15 cm column to 100% EtOAc, fractions 152-186 afforded a crude yellow residue (0.89 g) which was further purified by chromatography with $CHCl_3$ to give a yellow powder (92 mg). Purification of a portion of this powder (13 mg) by flash chromatography (2% MeOH/C_6H_6) gave **2** (3.6 mg) followed by **4** (8.6 mg). The large Si gel column was finally eluted with MeOH to give a residue (6.64 g) which was not investigated.

(−)-DIHYDROPARTHENOLIDE [**1**].—Tlc (2% MeOH/C_6H_6) Rf 0.50, $[\alpha]^{19}_D$ −57° (*c* 1.4, $CHCl_3$), lit. (8) $[\alpha]^{26}_D$ −62° ($CHCl_3$); ir ν max (CCl_4) 1775, 1650, 1450, 980 cm^{-1}; ^{1}H and ^{13}C nmr, see Tables 1 and 2, respectively; eims *m/z* (rel. int.) 250 (M^+,2), 232 (3), 207 (4), 192 (13), 133 (19), 119 (32).

(−)-PARTHENOLIDE [**2**].—Tlc (2% MeOH/C_6H_6) Rf 0.42, (MeOH-EtOAc-petrol, 1:3:6) Rf 0.57; $[\alpha]^{20}_D$ −78° ($CHCl_3$), lit. (34) $[\alpha]^{20}_D$ −81.4° ($CHCl_3$); ir ν max (CCl_4) 3020, 2920, 1770, 1650, 1281, 1260, 1130, 940 cm^{-1}; ^{1}H nmr ($CDCl_3$), see Table 1; eims *m/z* (rel. int.) 248 (M^+,2), 230 (9), 191 (25), 190 (61), 119 (100).

(−)-BISPARTHENOLIDINE [**3**].—Mp 100-103° ($CHCl_3$); tlc (MeOH-EtOAc-petrol, 1:3:6) Rf 0.39; $[\alpha]^{20}_D$ −112° ($CHCl_3$); ir ν max (CCl_4) 3365, 3020, 2920, 1770, 1480, 1215, 1175, 1000, 940 cm^{-1}; ^{1}H and ^{13}C nmr, see Tables 1 and 2, respectively; eims *m/z* (rel. int.) 513 (M^+,8) 278 (100), 264 (14); hrms (composition interpret., calcd. millimass) 513.3077 ($C_{30}H_{43}NO_6$, M^+, 513.3090), 278.1752 ($C_{16}H_{24}NO_3$, M-$C_{14}H_{19}O_3$, 278.1756), 264.1600 ($C_{15}H_{22}NO_3$, M-$C_{15}H_{21}O_3$, 264.1594).

LIRIODENINE [**4**].—Mp 278-281° (dec.), lit. (35) mp 282°; tlc (MeOH-EtOAc-petrol, 1:3:6) Rf 0.22; ^{1}H nmr (10% DMSO-d_6/$CDCl_3$) δ 6.41 (s, OCH_2O), 7.21 (s, H-3), 7.58 (t, *J*=8.0 Hz, H-9), 7.77 (t, *J*=8.0 Hz, H-10), 7.83 (br s, H-4), 8.57 (d, *J*=8.0 Hz, H-8), 8.72 (d, *J*=8.0 Hz, H-11), 8.90 (br s, H-5); ^{13}C nmr, same a previously reported (27); eims *m/z* (rel. int.) 275 (M^+,80), 247 (14), 246 (10).

CYTOTOXICITY ASSAY.—The KB cell culture assay was performed at the National Cancer Institute in Bangkok using a previously reported protocol (36), and the ED_{50} for **3** was found to be 0.60 μg/ml in each of two duplicate determinations.

ACKNOWLEDGMENTS

G.L.L. acknowledges financial support from the Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

1. N. Ruangrungsi, A. Aukkanibutra, T. Phadungcharoen, G.L. Lange, and M. Lee, *J. Sci. Soc. Thailand,* submitted.
2. J.C. Willis, "A Dictionary of Flowering Plants and Ferns," 7th edition, revised by H.K. Airy Shaw, University Press, Cambridge, 1966, p. 832.
3. H. Keng, in: "Flora of Thailand," vol. 2, part 3, ASRCT Press, Bangkok, 1975, pp. 266-267.
4. T. Smitinand, "Thai Plant Names (Botanical Names—Vernacular Names)," Funny Publishing Ltd., Bangkok, 1980, pp. 33, 252.
5. L.M. Perry, "Medicinal Plants of East and Southeast Asia," MIT Press, Cambridge, Mass., 1980, pp. 250-253.
6. I.H. Burkill, "A Dictionary of the Economic Products of the Malay Peninsula," Vol. II, Ministry of Agriculture and Co-operatives, Kuala Lumpur, 1966, pp. 1489-1491.
7. J.L. Hartwell, *Lloydia,* **33**, 97 (1970).
8. T.R. Govindachari, B.S. Joshi, and V.N. Kamat, *Tetrahedron,* **21**, 1509 (1965).
9. M. Ogura, G.A. Cordell, and N.R. Fransworth, *Phytochemistry,* **17**, 957 (1978).
10. S.K. Talapatra, A. Patra, and B. Talapatra, *J. Chem. Soc., Chem. Commun.,* 1534 (1970).
11. N.H. Fischer, Y.F. Wu-Shih, G. Chiari, F.R. Fronczek, and S.F. Watkins, *J. Nat. Prod.,* **44**, 104 (1981).
12. M. Budesinsky, D. Saman, G. Nowak, B. Drozdz, and M. Holub, *Coll. Czech. Chem. Commun.,* **49**, 637 (1984).
13. F.S. El-Feraly, Y.M. Chan, and G.A. Capiton, *J. Org. Chem.,* **44**, 3952 (1979).
14. A. Quick and D. Rogers, *J. Chem. Soc., Perkin II,* 465 (1976).
15. A.S. Bawdekar, G.R. Kelkar, and S.C. Battacharya, *Tetrahedron Lett.,* 1225 (1966).
16. S. Macura, K. Wüthrich, and R.R. Ernst, *J. Magn. Reson.,* **46**, 269 (1982).
17. M. Yanagita, S. Inayama, T. Kawamata, and T. Okura, *Tetrahedron Lett.,* 2073 (1969).
18. I.R.C. Bick, J.W. Gillard, and M.M. Leow, *Phytochemistry,* **25**, 972 (1986).
19. J.W. Steele, J.B. Stenlake, and W.D. Williams, *J. Chem. Soc.,* 2627 (1959).
20. J. Harmatha and Z. Samek, *Coll. Czech. Chem. Commun.,* **47**, 2779 (1982).
21. P.D.D.B. Lima, M. Garcia, and J.A. Rabi, *J. Nat. Prod.,* **48**, 986 (1985).
22. I.R.C. Bick, J.H. Bowie, and G.K. Douglas, *Aust. J. Chem.,* **20**, 1403 (1967).
23. W.I. Taylor, *Tetrahedron,* **14**, 42 (1961).
24. E.E. Dickey, *J. Org. Chem.,* **25**, 1389 (1960).
25. I.R.C. Bick and C.K. Douglas, *Tetrahedron Lett.,* 1629 (1964).
26. S.M. Kupchan, M.I. Suffness, and E.M. Gordon, *J. Org. Chem.,* **35**, 1682 (1970).
27. H. Guinaudeau, M. Leboeuf, and A. Cavé, *J. Nat. Prod.,* **46**, 761 (1983).
28. H. Guinaudeau, M. Leboeuf, and A. Cavé, *J. Nat. Prod.,* **42**, 325 (1979).
29. H. Guinaudeau, M. Leboeuf, and A. Cavé, *Lloydia,* **38**, 275 (1975).
30. K.H. Lee, E.S. Huang, C. Piantadosi, J.S. Pangano, and T.A. Geissman, *Can. Res.,* **31**, 1649 (1971).
31. D. Warthen, E.L. Gooden, and M. Jacobson, *J. Pharm. Sci.,* **58**, 637 (1969).
32. C.D. Hufford, M.J. Funderback, J.M. Morgan, and L.W. Robertson, *J. Pharm. Sci.,* **64**, 789 (1975).
33. I.H. Hall, K.H. Lee, C.O. Starnes, Y. Sumida, R.Y. Wu, T.G. Waddell, J.W. Cochran, and K.G. Gerhart, *J. Pharm. Sci.,* **68**, 537 (1979).
34. M. Soucek, V. Herout, and F. Sorm, *Coll. Czech. Chem. Commun.,* **26**, 803 (1961).
35. M.A. Buchanan and E.E. Dickey, *J. Org. Chem.,* **25**, 1389 (1960).
36. R.I. Geran, N.G. Greenberg, M.N. MacDonald, A.M. Schumacher, and B.J. Abbott, "Cancer Chemotherapy Reports," Part 3, Vol. 3, Drug Evalution Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD, 1972, p. 17.

Received 4 March 1987