A New Modified Steroid, Hancopregnane, and a New Monoterpene from Cynanchum hancockianum

Yaeko Konda, Yumiko Toda, Hiroaki Takayanagi, Haruo Ogura,
Yoshihiro Harigaya, Hongxiang Lou, Xian Li, and Masayuki Onda

J. Nat. Prod., **1992**, 55 (8), 1118-1123• DOI:
10.1021/np50086a013 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 8, pp. 1118-1123, August 1992

A NEW MODIFIED STEROID, HANCOPREGNANE, AND A NEW MONOTERPENE FROM *CYNANCHUM HANCOCKIANUM*

YAEKO KONDA, YUMIKO TODA, HIROAKI TAKAYANAGI, HARUO OGURA, YOSHIHIRO HARIGAYA,*

School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108, Japan

HONGXIANG LOU,[1] XIAN LI,* and MASAYUKI ONDA

Shenyang College of Pharmacy, Wenhua-lu, Shenyang, China

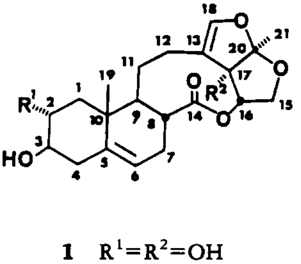
ABSTRACT.—A new modified steroid, hancopregnane [**1**], and a new monoterpene **2** have been isolated from *Cynanchum hancockianum* along with a number of known compounds. Their structures are elucidated by means of spectroscopic (X-ray and/or nmr) analysis.

We previously reported two antitumor alkaloids, antofine [**8**] and 6-*O*-demethylantofine [**9**], from the HCl-soluble portion of *Cynanchum hancockianum* (Maxim.) Al. Iljinski. (Asclepiadaceae) grown in Inner Mongolia (1). Because of biological interest, investigations have been continued leading to the isolation of two new compounds **1** and **2** along with a number of known compounds. This paper deals with the structure elucidation of these compounds by X-ray and/or nmr analysis.

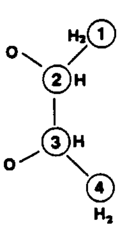
RESULTS AND DISCUSSION

Compound **1**, hancopregnane, $C_{21}H_{28}O_7$, gave a reddish purple coloration with 15% H_2SO_4/EtOH. The ir spectrum showed hydroxyl (3560, 3380 cm^{-1}) and ester carbonyl bands (1723 cm^{-1}).

DEPT experiments indicated two tertiary methyl, six methylene, seven methine,

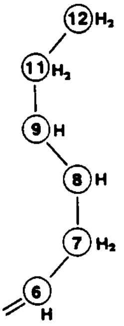


1 $R^1=R^2=OH$
3 $R^1=OH, R^2=H$
4 $R^1=R^2=H$

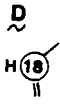


A

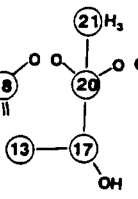
E



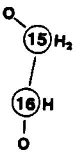
B



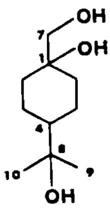
D



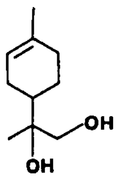
F



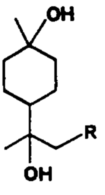
C



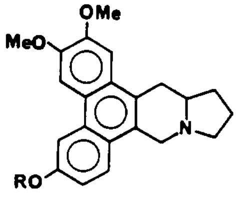
2



5



6 R=OH
7 R=H



8 R=Me
9 R=H

---

[1] Present address, Shandong Medical University, Jinan City, Shandong, China.

and five quaternary carbons which were unambiguously assigned by 2D nmr spectroscopy (Table 1). ^{1}H-^{1}H COSY experiments indicated the presence of four independent spin systems, **A**, **B**, **C**, and **D**. Judging from the proton and carbon resonances, the following carbons contained *O* functions: two methines (δ_H 3.70, δ_C 72.22, C-2; δ_H 3.35, δ_C 76.17, C-3) in system **A**; a methylene (δ_H 4.18, 3.84, δ_C 66.48, C-15) and a methine (δ_H 5.37, δ_C 83.14, C-16) in system **C**; and three quaternary carbons [δ_C 91.20, C-17; δ_C 116.23, C-20 (or δ_C 119.63, C-13) ($O \times 2$); δ_C 178.24, C-14]. Furthermore, a methine (δ_H 5.46, δ_C 120.81, C-6) in system **B** was attributable to a trisubstituted olefin, and a methine (δ_H 6.41, δ_C 145.88, C-18) in system **D** to a trisubstituted olefin with an *O* function.

TABLE 1. Nmr Data for **1**.[a]

Position	δ_C	Correlated H[b] δ_H	C coupled with H[c]	H coupled with H[d]
1	44.08 t	Hα-1 1.09 t (13)	C-2, C-9, C-19	Hβ-1, H-2
		Hβ-1 2.21 dd (13,5)		Hα-1, H-2
2	72.22 d	H-2 3.70 ddd (13,8,5)		H_2-1, H-3
3	76.17 d	H-3 3.35 m		H-2, H_2-4
4	38.85 t	Hα-4 2.38 dd (14,5)	C-2, C-3, C-5 C-6, C-10	H-3, Hβ-4
		Hβ-4 2.26 dt (2,14)	C-2, C-3, C-5 C-10	H-3, Hα-4
5	138.95 s			
6	120.81 d	H-6 5.46 brd (5)	C-4, C-10	H_2-7
7	29.47 t	H_2-7 2.10 m	C-6	H-8
8	40.08 d	H-8 2.46 m (9)	C-6, C-9, C-10	H_2-7, H-9
9	52.79 d	H-9 1.34 m	C-5, C-10	H-8, H_2-11
10	39.95 s			
11	20.52 t	H_A-11 2.53 dt (3,14)		
		H_B-11 1.43 tt (14,2)	C-13	H-9, H_2-12
12	27.63 t	H_2-12 2.05 m		H_2-11
13	119.63 s			
14	178.24 s			
15	66.48 t	H_A-15 4.18 dd (9,7)	C-13, C-17	H_B-15, H-16
		H_B-15 3.84 dd (10,9)	C-16	H_A-15, H-16
16	83.14 d	H-16 5.37 dd (10,7)		H_2-15
17	91.20 s			
18	145.88 d	H-18 6.41 s	C-13, C-17, C-20	
19	19.12 q	H_3-19 0.97 s	C-1, C-5, C-9, C-10	
20	116.23 s			
21	19.98 q	H_3-21 1.48 s	C-15, C-17, C-20	
		HO-17 4.11 s	C-13, C-17	

[a] Spectra were taken on a Varian XL-400 (^{1}H, 400 MHz; ^{13}C, 100.6 MHz).

[b] ^{1}H-^{13}C (one-bond) COSY. Figures in parentheses are coupling constants (Hz).

[c] HMBC.

[d] ^{1}H-^{1}H COSY.

HMBC experiments correlated two quaternary carbons (δ_C 138.95, C-5; δ_C 39.95, C-10) to a methyl (δ_H 0.97, H_3-19), showing the presence of a propyl fragment **E**. A combination of systems **A** and **B** with **E** was proved on the basis of the correlations of a methylene (δ_C 44.08, C-1) and a methine (δ_C 52.79, C-9) to H_3-19, and C-5, C-6, and C-10 to a methylene (δ_H 2.38, Hα-4) to form a 10-methyl-Δ^5-decalin possessing 2,3-di-*O* functions oriented trans-diequatorially ($J_{2,3}=8$ Hz) and a 9-ethylene chain. Numbering refers to that of steroids.

The presence of a butyl fragment **F** was ascertained by HMBC correlations of C-17 and C-20 to a methyl (δ_H 1.48, H_3-21), and C-13 and C-17 to a hydroxyl (δ_H 4.11, 17-OH). The correlations of C-13, C-17, and C-20 to H-18, and of C-17 to H_A-15 (δ_H 4.18) led to the formation of a 2,3,3a,6a-tetrahydro-3a-hydroxy-6a-methylfuro[2,3-*b*]furan combining systems **C** and **D** with **F**.

A methylene (δ_H 2.05, δ_C 27.63, C-12) adjacent to C-13 was deduced from the correlation of C-13 to a methylene (δ_H 1.43, H_B-11). If it is assumed that the 2,3-di-*O* functions are hydroxyls and that C-8 and C-16 are connected by a lactone composed of the one remaining quaternary carbon (C-14) and two oxygen atoms, a 15,20:18,20-diepoxy-13,14:14,15-disecopregnane is the result. The carbon resonances of **1** were similar to those of glaucogenin A [**3**] (2), except for those of the furofuran moiety. The downfield shifts of ring carbons were explainable by the effect of 17-OH. The upfield shift (5.5 ppm) of C-21 suggested it to be cis to 17-OH.

An X-ray analysis proved the gross structure of **1** deduced above to be correct (Figure 1 and Table 2). In accord with the nmr analysis, it was found that 2α-OH and 3β-OH are trans-diequatorial, and 17α-OH and 20α-Me (H_3-21) are cis. Furthermore, H-8, H-9, and H-16 were found to be β, α, and β, respectively. As for the nine-membered ring conformation in the solid state, the twisted boat chair form with symmetry D_3 (3) and the twisted chair boat form with symmetry C_2 (4,5) are known. X-ray analysis showed a deformed conformation with no symmetry element for the nine-membered ring of **1**. Thus, **1** was established as 15,20α:18,20β-diepoxy-2α,3β,17α-trihydroxy-13,14:14,15-disecopregna-5,13(18)-dien-14-oic acid 16-oxylactone.

Compound **2** gave a red coloration with 15% H_2SO_4/EtOH. The formula $C_{10}H_{20}O_3$ was determined by eims and and elemental analysis, showing **2** to be a monocyclic compound on the basis of its degree of unsaturation (= 1). The ir spectrum showed a hydroxyl band (3350 cm^{-1}). DEPT experiments indicated the presence of two tertiary methyl, five methylene, one methine, and two quaternary carbons. Judging from the proton and carbon resonances and proton splitting multiplicity, a hydroxylated methylene (δ_H 3.57, 3.43, each d, J_{gem} = 18.8 Hz, δ_C 68.57, C-7) bonded to a

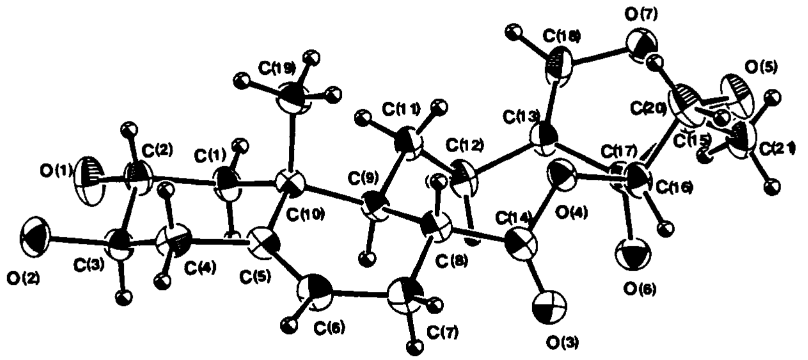


FIGURE 1. Molecular structure of **1**.

TABLE 2. Atomic Parameters (× 10^4) of **1**.

Atom	x	y	z	Beq/Å^{2a}
O-1A	0.7423	0.6882	0.9999	5.7(3)
O-2A	0.6129(6)	0.6012(5)	1.1750(8)	5.2(3)
O-3A	0.7176(6)	1.1720(5)	1.8753(8)	5.1(3)
O-4A	0.5805(6)	1.2783(5)	1.6838(8)	3.5(2)
O-5A	0.6141(7)	1.5337(5)	1.6795(8)	5.7(3)
O-6A	0.8733(6)	1.2908(5)	1.7177(7)	4.4(3)
O-7A	0.6815(7)	1.4411(5)	1.3881(8)	5.4(3)
C-1A	0.6811(7)	0.8590(6)	1.223(1)	3.7(3)
C-2A	0.6462(7)	0.7590(6)	1.129(1)	3.9(4)
C-3A	0.6455(7)	0.6952(6)	1.268(1)	3.8(4)
C-4A	0.5453(8)	0.7734(6)	1.407(1)	4.2(4)
C-5A	0.5716(7)	0.8777(6)	1.498(1)	3.5(3)
C-6A	0.5767(8)	0.9107(6)	1.676(1)	4.3(4)
C-7A	0.5953(8)	1.0155(7)	1.776(1)	4.6(4)
C-8A	0.5826(7)	1.0954(6)	1.655(1)	3.5(3)
C-9A	0.6522(7)	1.0268(5)	1.476(1)	3.0(3)
C-10A	0.5880(7)	0.9448(6)	1.373(1)	3.0(3)
C-11A	0.6607(8)	1.1031(6)	1.359(1)	3.7(4)
C-12A	0.7782(8)	1.1378(6)	1.412(1)	3.9(3)
C-13A	0.7464(8)	1.2622(6)	1.439(1)	3.5(3)
C-14A	0.6357(7)	1.1827(6)	1.755(1)	3.7(3)
C-15A	0.5378(8)	1.4740(6)	1.704(1)	5.5(4)
C-16A	0.6384(8)	1.3617(6)	1.739(1)	3.7(3)
C-17A	0.7522(7)	1.3350(6)	1.621(1)	3.5(3)
C-18A	0.7057(9)	1.3261(6)	1.320(1)	5.0(5)
C-19A	0.4518(8)	1.0104(6)	1.287(1)	4.7(4)
C-20A	0.7214(8)	1.4532(6)	1.576(1)	4.1(4)
C-21A	0.829(1)	1.4948(7)	1.605(1)	5.4(5)
O-1B	0.2171(7)	0.1297(5)	0.8178(8)	4.8(3)
O-2B	0.0975(6)	0.1780(5)	1.1506(8)	5.1(3)
O-3B	−0.0417(6)	−0.3536(5)	0.4041(8)	5.0(3)
O-4B	0.1665(6)	−0.4741(4)	0.4542(7)	3.3(2)
O-5B	0.2905(6)	−0.6981(5)	0.0754(8)	4.5(3)
O-6B	0.0464(6)	−0.4525(5)	0.0251(8)	4.6(3)
O-7B	0.3892(6)	−0.5875(5)	0.0205(8)	5.2(3)
C-1B	0.1849(7)	−0.0434(6)	0.724(1)	3.5(3)
C-2B	0.2015(7)	0.0444(6)	0.884(1)	3.5(3)
C-3B	0.0850(8)	0.0927(6)	1.002(1)	4.0(4)
C-4B	0.0759(8)	−0.0021(6)	1.068(1)	4.3(4)
C-5B	0.0689(7)	−0.0973(6)	0.919(1)	3.3(3)
C-6B	−0.0231(8)	−0.1366(6)	0.909(1)	4.0(4)
C-7B	−0.0381(7)	−0.2323(6)	0.772(1)	4.1(4)
C-8B	0.0846(7)	−0.2989(6)	0.663(1)	3.1(3)
C-9B	0.1328(7)	−0.2167(5)	0.609(1)	2.8(3)
C-10B	0.1737(7)	−0.1455(6)	0.779(1)	2.9(3)
C-11B	0.2383(7)	−0.2769(6)	0.467(1)	3.6(3)
C-12B	0.1856(8)	−0.2981(6)	0.270(1)	4.3(4)
C-13B	0.2408(8)	−0.4192(6)	0.169(1)	3.7(4)
C-14B	0.0589(7)	−0.3757(6)	0.496(1)	3.4(3)
C-15B	0.2766(8)	−0.6573(6)	0.265(1)	4.8(4)
C-16B	0.1576(7)	−0.5441(6)	0.280(1)	3.4(3)
C-17B	0.1683(7)	−0.4970(6)	0.121(1)	3.4(3)
C-18B	0.3607(8)	−0.4761(7)	0.110(1)	4.9(4)
C-19B	0.3063(7)	−0.2190(6)	0.860(1)	3.8(3)
C-20B	0.2680(7)	−0.6048(6)	−0.001(1)	3.6(3)
C-21B	0.2319(8)	−0.6294(6)	−0.196(1)	5.0(4)
O-8	0.9104(7)	0.3830(6)	1.072(1)	7.0(4)
O-9	0.0817(7)	0.2033(6)	0.5122(9)	7.2(4)

$^{a}B_{eq} = 4/3\Sigma_i\Sigma_j\beta_{ij}\alpha_i\alpha_j$.

quaternary carbon (δ_C 70.82, C-1) possessing a hydroxyl was present. Also, another quaternary carbon (δ_C 74.41, C-8) possessed a hydroxyl. These observations indicated **2** to be a cyclohexane possessing tertiary hydroxyl, hydroxymethyl, and 1-hydroxy-1-methylethyl groups. The comparisons with the ^{1}H and ^{13}C resonances of *p*-menthane-1,8,9-triol [**6**] (6) and with a related compound, *trans*-terpin [**7**] (7), led to *p*-menthane-1,7,8-triol as the structure for **2**. Finally, because of the isolation under the acidic conditions, there is a possibility that all of these compounds exist in nature as glycosides.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) were determined on a micro hot-stage apparatus. Specific rotations were taken on a JASCO DPI-181 polarimeter. Spectra were recorded on the following spectrometers: ir, Hitachi 260-30; ^{1}H nmr, Varian XL-400 (400 MHz), Bruker-Ac (E)-250 (250 MHz) (reference TMS); ^{13}C nmr, Varian XL-400 (100.6 MHz), Bruker-Ac(E)-250 (62.9 MHz) (reference TMS); eims and hrms, JEOL JMS DX-300; elemental analysis, Perkin-Elmer 240B.

All nmr spectra were taken at 20° in $CDCl_3$ using a 5-mm tube. 2D nmr and DEPT experiments were performed using Varian's standard pulse sequences.

EXTRACTION AND ISOLATION.—The roots of *C. hancockianum* were collected in Inner Mongolia. Plant material was identified by Prof. Y. Guo, Shenyang College of Pharmacy, and a voucher specimen is deposited in the Herbarium of Shenyang College of Pharmacy.

The air-dried, powdered plant material (40 kg) was extracted with boiling EtOH (40 liters × 4) for 5 h to yield the EtOH extract (6.5 kg), one-half of which was dissolved in 0.5 N HCl (6 liters) and filtered. The acidic solution was made alkaline (pH 9) with concentrated NH_4OH and extracted with $CHCl_3$ (6 liters × 4) to yield the $CHCl_3$ extract (150 g).

The $CHCl_3$ extract (80 g) was chromatographed over Si gel (300 g) eluting with a mixture of petroleum ether and Me_2CO of increasing polarity. The first elution, petroleum ether-Me_2CO (97:3$\mapsto$90:10) (25 liters), afforded 1-*p*-menthene-8,9-diol [**5**] (8,9) (18 mg), 4-hydroxy-3-methoxyacetophenone (24 mg), glaucogenin C [**4**] (10) (100 mg). Second elution (80:20) (40 liters) gave **3** (2) (120 mg), antofine [**8**] (1) (280 mg), and the crude product (3.2 g) which was rechromatographed over Si gel (40 g) eluting with $CHCl_3$-MeOH (95:5) (25 liters) to afford **1** (14 mg), **2** (18 mg), and **6** (6) (50 mg). A third elution with petroleum ether-Me_2CO-MeOH (4:6:1) (5 liters) gave 6-*O*-demethylantofine [**9**] (1) (52 mg). The known compounds were identified by nmr analysis or direct comparisons with authentic samples.

Hancopregnane [**1**].—Colorless needles: mp 202–203° (Me_2CO); $[\alpha]^{23}D$ +3.75° ($c = 0.16$, MeOH); ir ν max ($CHCl_3$) cm^{-1} 3560, 3380, 1723; ^{1}H and ^{13}C nmr see Table 1; hrms m/z $[M]^+$ 392.1848 (392.1836 for $C_{21}H_{28}O_7$). *Anal.* calcd for $C_{21}H_{28}O_7$, C 64.27, H 7.19; found C 64.34, H 7.24.

p-*Menthane-1,7,8-triol* [**2**].—Colorless prisms: mp 149–151° ($CHCl_3$); ir ν max (KBr) cm^{-1} 3340; ^{1}H nmr (250 MHz) δ 3.75, 3.42 (each 1H, d, $J = 10.8$ Hz, H_2-7), 1.9–1.3 (9H, m), 1.24, 1.11 (each 3H, s, H_3-9, H_3-10); ^{13}C nmr (62.9 MHz) δ 74.41 (s, C-8), 70.82 (s, C-1), 68.57 (t, C-7), 44.19 (d, C-4), 40.34 (t, C-2, C-4), 25.32, 25.19 (each q, C-9, C-10), 20.35 (t, C-3, C-6); eims m/z $[M]^+$ 188. *Anal.* calcd for $C_{10}H_{20}O_3$, C 63.79, H 10.70; found C 63.65, H 10.76.

Glaucogenin A [**3**].—Colorless needles: mp 227–228.5° (Me_2CO) [lit. (2) mp 225–231°]; eims m/z $[M]^+$ 376 ($C_{21}H_{28}O_6$).

Glaucogenin C [**4**].—Colorless needles: mp 208–209° (Me_2CO) [lit. (10) mp 205–206°]; eims m/z $[M]^+$ 360 ($C_{21}H_{28}O_5$).

1-p-*Menthene-8,9-diol* [**5**].—Colorless needles: mp 50–52° (Me_2CO) [lit. (8,9) 4*R*,8*R*, mp 52–53°; 4*R*,8*S*, mp 64.5–66°]; eims m/z $[M-18]^+$ 152. *Anal.* calcd for $C_{10}H_{18}O_2$, C 70.55, H 10.66; found C 70.34; H 10.71.

p-*Menthane-1,8,9-triol* [**6**].—Colorless needles, mp 115–116° (Me_2CO) [lit. (6) mp 118–118.5° (EtOAc)]; eims m/z $[M-H_2O-CH_2OH]^+$ 139. *Anal.* calcd for $C_{10}H_{20}O_3$, C 63.79, H 10.74; found C 63.65, H 10.76.

X-RAY CRYSTALLOGRAPHIC DATA FOR **1**.[2]—$C_{21}H_{28}O_7 \cdot 2H_2O$, formula weight = 428.48, tri-

---

[2] Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

clinic, space group *P1*; $a = 11.190$ (1), $b = 13.221$ (1), $c = 7.656$ (8) Å; $D_{calcd} = 1.427$ g·cm^{-3}, μ (CuKα) = 8.87 cm^{-1}, Z = 2, 3119 independent reflections were measured out of 2θ max = 139.9° with a Rigaku AFC5R four cycle diffractmeter using CuKα radiation (λ = 1.54178 Å) with an incident beam graphite monochromator. The data were collected at 296K from a colorless prism (0.3 × 0.3 × 0.2 mm) using the ω–2θ scan technique.

The structure was solved by the direct method (MITHRIL) (11) and refined by the full-matrix least-squares method. The positions of all hydrogen atoms were calculated. Two crystallographically independent molecules existing in an asymmetric unit were represented by the suffix letters A and B.

The absolute configuration was determined by the anomalous scattering technique. When the refinements were carried out by use of the atomic parameters obtained from the result of the direct method, the residual values converged to R = 0.049 and Rw = 0.053.

The atomic scattering factors and anomalous scattering corrections were taken from *International Tables for X-ray Crystallography* (12). The final atomic parameters are listed in Table 2. All calculations were performed using the TEXSAN (13) crystallographic software package of Molecular Structure Corporation.

LITERATURE CITED

1. X. Li, J. Peng, M. Onda, Y. Konda, M. Iguchi, and Y. Harigaya, *Heterocycles,* **29**, 1797 (1989).
2. T. Nakagawa, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.,* **31**, 870 (1983).
3. P. Groth, *Acta Chem. Scand.,* **23**, 1311 (1969).
4. R.F. Bryan and J.D. Dunitz, *Helv. Chim. Acta,* **43**, 3 (1960).
5. S. Dahl and P. Groth, *Acta Chem. Scand.,* **25**, 1114 (1971).
6. T. Suga, Y.S. Lee, and T. Hirata, *Bull. Chem. Soc. Jpn.,* **56**, 784 (1983).
7. L.-G. Yi, C.D. Bates, A.I. Gray, and P.G. Waterman, *Planta Med.,* 368 (1988).
8. R.M. Carman, K.L. Greenfield, and W.T. Robinson, *Aust. J. Chem.,* **39**, 21 (1986).
9. R.M. Carman, J.J. De Voss, and K.L. Greenfield, *Aust. J. Chem.,* **39**, 441 (1986).
10. Z.-X. Zhang, J. Zhou, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.,* **33**, 1507 (1985).
11. C.J. Gilmore, *J. Appl. Crystallogr.,* **17**, 42 (1984).
12. "International Tables for X-ray Crystallography," Kynoch Press, Birmingham, 1974, Vol. IV.
13. TEXSAN-TEXRAY Structure Analysis Package, Molecular Structure Corporation, 1985.

Received 29 January 1992