Metabolites of the Dorid Nudibranch *Chromodoris sedna*

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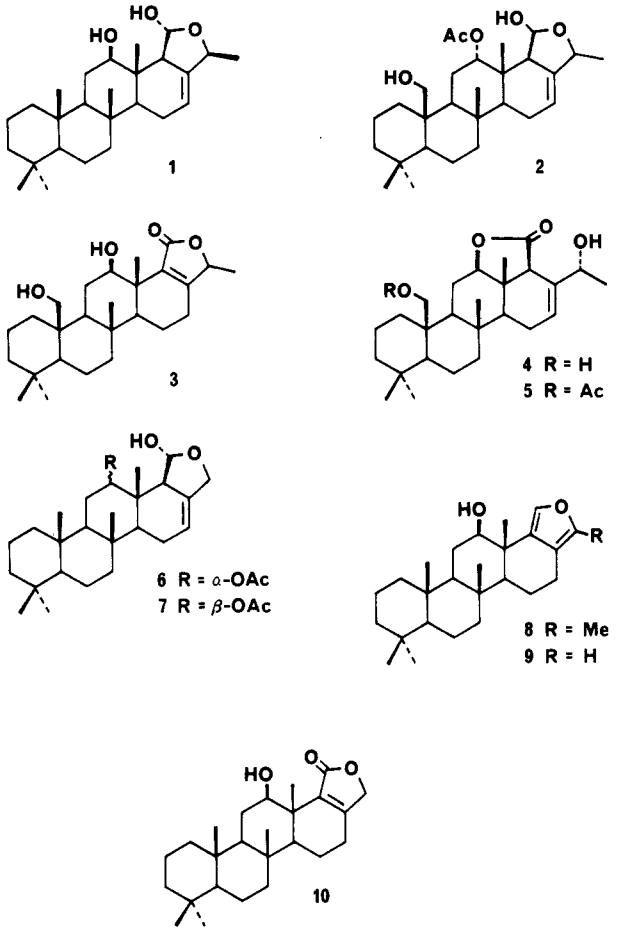
Five C_{26} tetracyclic terpenes have been isolated from the dorid nudibranch *Chromodoris sedna*. The structure of sednolide (4) was determined by single-crystal X-ray analysis. The structures of 12-deacetyl-20-methyl-12-epideoxoscalarin (1), 23-hydroxy-20-methyldeoxoscalarin (2), 23-hydroxy-20-methylscalarolide (3), and sednolide 23-acetate (5) were determined by analysis of spectral data.

Dorid nudibranchs are shell-less marine molluscs that appear to employ a chemical defense mechanism against predation.[1] The chemicals found in their defensive secretions are generally identical with or similar to the metabolites of sponges on which the dorids feed. As part of a systematic study of the metabolites from nudibranchs,[2] we have isolated a number of C_{26} tetracyclic terpenes from the nudibranch *Chromodoris sedna* Marcus and Marcus 1976. In this paper we describe the structural elucidation of five new C_{26} tetracyclic terpenes. ^1H NMR spectra suggested that the nudibranchs contained additional terpenoid materials, but these could not be isolated in sufficient quantity or purity to allow identification.

12-Deacetyl-20-methyldeoxoscalarin (1, Chart I) obtained as a noncrystalline solid, has the molecular formula $C_{26}H_{42}O_3$. Like most hemiacetals, the diol 1 did not give a molecular ion in the mass spectrum, the highest peak being that at m/z 384 due to loss of water from the molecular ion. The infrared spectrum contained a hydroxyl band at 3420 cm^{-1} (broad) but lacked carbonyl bands. The ^1H NMR spectrum contained methyl signals at δ 0.75 (s, 3 H), 0.81 (s, 3 H), 0.84 (s, 3 H), 0.86 (s, 3 H), and 0.92 (s, 3 H), reminiscent of the spectra of scalarins. A sixth signal at δ 1.18 (d, 3 H, J = 7 Hz) was assigned to a methyl group at C-20 by analogy with the C-26 tetracyclic terpenes found in sponges.[3] The methyl signal was coupled to a methine proton signal at δ 4.55 (br q, 1 H, J = 7 Hz) that shows allylic coupling to a vinyl proton signal at 5.34 (br s, 1 H, $w_{1/2}$ = 10 Hz). A methine proton signal at δ 2.20 (m, 1 H) was coupled to an acetal proton signal at δ 5.28 (d, 1 H, J = 6 Hz) and to the protons at δ 4.55 and 5.34, through W coupling and allylic coupling, respectively. A signal at δ 3.40 (dd, 1 H, J = 11, 5 Hz) was assigned to an axial proton on a carbon bearing a hydroxyl. Two exchangeable proton signals at δ 3.12 and 3.64 were assigned to the hydroxyl protons. Comparison of these data with the ^1H NMR data for deoxyscalarin (6) and 12-epideoxoscalarin (7)[4] strongly supported the proposed structure for diol 1 as did comparison of the ^{13}C NMR spectra (Table I). The stereochemistry of the methyl group at C-20 was defined by the presence of W coupling between the protons at C-18 and C-20, indicating a syn relationship.

Treatment of the diol 1 with acetic anhydride in pyridine at 25 °C for 16 h gave the furan 8 in good yield. The ^1H NMR spectrum contained a furan proton signal at δ 7.40 (s, 1 H) and a methyl signal at δ 2.13 (s, 3 H). The ^1H NMR spectrum was remarkably similar to that of furan 9, obtained by lithium aluminum hydride reduction of scalarolide,[5] in which the C-19 furan proton signal appeared at δ 7.39 (s, 1 H) with the C-20 furan proton signal at δ 6.91 (s, 1 H).

Chart I



23-Hydroxy-20-methyldeoxoscalarin (2) is the least well-defined of the metabolites. The molecular formula $C_{28}H_{44}O_5$ was obtained by assuming that the highest peak in the mass spectrum at m/z 442 was due to loss of water from the molecular ion. The infrared spectrum indicated the presence of hydroxyl (3560 cm^{-1}) and acetate (1730 cm^{-1}) groups. The ^1H NMR spectrum of 2 showed significant similarities to that of deoxoscalarin (6):[4] the signal

(1) Thompson, J. E.; Walker, R. P.; Wratten, S. J.; Faulkner, D. J. *Tetrahedron* **1982**, *38*, 1865 and references cited therein.

(2) Hochlowski, J. E.; Faulkner, D. J. *Tetrahedron Lett.* **1981**, *22*, 271. Walker, R. P.; Faulkner, D. J. *J. Org. Chem.* **1981**, *46*, 1475. Hochlowski, J. E.; Walker, R. P.; Ireland, C.; Faulkner, D. J. *Ibid.* **1982**, *47*, 88.

(3) (a) Kashman, Y.; Zviely, M. *Tetrahedron Lett.* **1979**, 3879. (b) Kazlauskas, R.; Murphy, P. T.; Wells, R. J.; Daly, J. J. *Aust. J. Chem.* **1980**, *33*, 1783. (c) Kazlauskas, R.; Murphy, P. T.; Wells, R. J. *Ibid.* **1982**, *35*, 51.

(4) Cimino, G.; De Stefano, S.; Minale, L.; Trivelone, E. *J. Chem. Soc., Perkin Trans. 1*, **1977**, 1587.

(5) Walker, R. P.; Thompson, J. E.; Faulkner, D. J. *J. Org. Chem.* **1980**, *45*, 4976.

0022-3263/83/1948-1738$01.50/0

Table I. Comparison of ^{13}C NMR Data for 12-Deacetyl-20-methyl-12-epideoxoscalarin (1) and 12-Epideoxoscalarin (7)

	7	1		7	1
C-1[a]	39.6	39.9	C-15	22.2	22.0
C-2	18.1[b]	18.1[f]	C-16	116.3	116.4
C-3	41.5[c]	41.6[g]	C-17	136.2	140.2
C-4	33.2	33.3	C-18	61.2	61.4
C-5	56.4	56.5	C-19	99.8	97.8
C-6	18.5[b]	18.6[f]	C-20	68.2	74.5
C-7	42.0[c]	42.1[g]	C-21	33.2	33.3
C-8	37.3[d]	37.4	C-22	21.4[e]	21.3
C-9	58.3	58.9	C-23	16.5	16.6[h]
C-10	37.5[d]	37.4	C-24	16.5	17.0[h]
C-11	23.5	25.9	C-25	9.8	8.7
C-12	82.7	81.2	C-26		19.3
C-13	37.7	39.9	OAc	171.4	
C-14	54.0	53.2	OAc	21.3[e]	

[a] See ref 4 for numbering system. [b-h] Assignments may be reversed.

at δ 5.34 (m, 1 H) was assigned to the C-16 olefinic proton (δ 5.36 in **6**), a signal at δ 5.21 (br s, 1 H) to the C-19 acetal proton (δ 5.12 in **6**), a signal at δ 4.89 (m, 1 H, $w_{1/2}$ = 4 Hz) to the C-12 proton (δ 4.82 in **6**), and the signal at δ 2.82 (m, 1 H) to the C-18 methine proton (δ 2.66 in **6**). The ^1H NMR spectrum also contained a signal at δ 1.26 (d, 3 H, J = 6 Hz) coupled to a signal at δ 4.70 (br q, 1 H, J = 6 Hz) due to protons at C-26 and C-20, respectively. Finally, one of the expected angular methyl signals has been replaced by signals at δ 4.03 (d, 1 H, J = 13 Hz) and 3.90 (d, 1 H, J = 13 Hz), assigned to a hydroxymethylene group at C-10 after comparison with spectral data for other 23-hydroxyscalarins. The structure proposed for compound **2** could not be confirmed due to lack of material.

23-Hydroxy-20-methylscalarolide (**3**), mp 278–9 °C, has the molecular formula $C_{26}H_{40}O_4$. The proposed structure **3** was based on comparison of spectral data with those of scalarolide (**10**).[5] The infrared bands at 3410 and 3315 cm^{-1} were assigned to hydroxyl groups while those at 1725 and 1660 cm^{-1} were assigned to the α,β-unsaturated γ-lactone in which interaction with hydroxyl group at C-12 caused the carbonyl band to be at abnormally low frequency, as had been observed in scalarolide (**10**). The UV absorption at 217 nm (ϵ 4725) was assigned to the unsaturated lactone. The ^{13}C NMR spectrum contained signals at δ 175.0, 135.4, 166.0, and 79.1 for the carbons of the butenolide ring; analogous signals for the scalarolide (**10**) were at δ 175.9, 135.8, 162.0, and 72.0. The ^1H NMR spectrum contained a methyl signal at δ 1.41 (d, 3 H, J = 6 Hz) coupled to a signal at δ 4.87 (q, 1 H, J = 6 Hz), indicating the presence of a methyl group at C-20. The spectrum also contained four angular methyl signals at δ 0.78, 0.86, 1.06, and 1.14, signals at δ 4.02 (d, 1 H, J = 12 Hz) and 3.86 (d, 1 H, J = 12 Hz) assigned to an angular hydroxymethylene group at C-10, a signal at δ 3.50 (dd, 1 H, J = 11, 5 Hz) assigned to the axial proton at C-12, and an exchangeable hydroxyl proton signal at δ 6.08 (s, 1 H). The ^{13}C NMR chemical shift of the hydroxymethylene carbon at δ 62.6 was almost identical with the literature[6] values (δ 62.3 and 62.9) for C-23 of a 23-hydroxyscalarin. The proposed structure for compound **3** was compatible with all spectral data.

Sednolide (**4**), mp 268–272 °C, has the molecular formula $C_{26}H_{40}O_4$. The infrared spectrum contained bands at 3400 and 1755 cm^{-1} assigned to hydroxyl and γ-lactone groups. The ^1H NMR spectrum contained signals at δ 0.77, 0.89, and 1.14 (all s, 3 H) assigned to angular methyl groups and

(6) See data for compound 17 in ref 3b and for compound 17 in ref 3c.

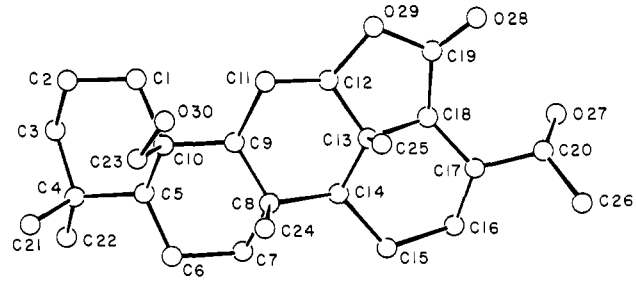


Figure 1. Computer-generated perspective drawing of sednolide (**4**). Hydrogens are omitted for clarity, and the absolute configuration is assumed.

at δ 3.96 (d, 1 H, J = 12 Hz) and 4.05 (d, 1 H, J = 12 Hz) due to an angular hydroxymethylene group. The remaining methyl signal at δ 1.35 (d, 3 H, J = 6 Hz) was coupled to a signal at δ 4.45 (br q, 1 H, J = 6 Hz) that was in turn coupled to an exchangeable proton signal at δ 3.50 (br, s, 1 H) and an olefinic proton at δ 5.52 (m, 1 H, J = 3, 2, 2 Hz). The olefinic signal at δ 5.52 was also coupled to a signal at δ 2.91 (br s, 1 H). The signal at δ 3.79 (dd, 1 H, J = 9, 6 Hz) was assigned to a pseudoaxial proton at C-12. Although examination of these data led to the proposal that sednolide (**4**) contained a γ-lactone ring between a carboxylate group at C-19 and an oxygen at C-12, this molecule was sufficiently different from scalarins described at that time to warrant confirmation by a single-crystal X-ray diffraction experiment.

Figure 1 is a computer-generated perspective drawing of the final X-ray model of sednolide (**4**). Hydrogens are omitted for clarity, and the absolute configuration shown was selected to make the hydroxymethyl substituent at C-10 have the β configuration. All of the cyclohexane rings are in the chair conformation, and the γ-lactone ring has an envelope conformation with a C-13 serving as the flap. The series of carbocyclic rings have a trans–anti arrangement. The configuration at C-20 is R^*.

The structure of the minor metabolite sednolide 23-acetate (**5**) was inferred solely from the ^1H NMR spectrum. The ^1H NMR spectrum of sednolide 23-acetate (**5**) was almost identical with that of sednolide except that it contained an acetate signal at δ 2.10 (s, 3 H) and the C-23 methylene proton signals had shifted to δ 4.67 (d, 1 H, J = 12 Hz) and 4.19 (d, 1 H, J = 12 Hz) from 4.09 and 3.96 in sednolide (**4**).

Specimens of the Australian dorid nudibranch *C. splendida* also contained a complex mixture of 24-methylscalarins as judged by ^1H NMR spectra. In Australian waters, 24-methylscalarins have been found in sponges of the genus *Phyllospongia*.[3b] Although it seems logical to suggest that the C_{26} terpenes from *C. sedna* were of dietary origin, we have not found a sponge source for these compounds. The fact that some batches of *C. sedna* did not contain scalarins, as judged by ^1H NMR spectroscopy, adds further credence to the dietary origin hypothesis. We had insufficient quantities of these compounds to perform valid feeding inhibition assays. Antimicrobial screening revealed that compounds 1 and 4 inhibited growth of the marine bacterium *Vibrio anguillarum* at 100 μg/disk.

Experimental Section[7]

Approximately 310 specimens of *C. sedna* were collected by hand by using SCUBA (−3 to −5 m) at Bahia de Concepcion, Baja California, Mexico in June 1980. The samples were stored in

(7) For general procedures, see: Frincke, J. M.; Faulkner, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 165.

acetone at ambient temperature for 2 weeks before the acetone was decanted and the solvent evaporated to obtain an aqueous suspension that was extracted with dichloromethane (3 × 100 mL). The combined extracts were dried over anhydrous sodium sulfate, and the solvent was evaporated to obtain an oily residue (900 mg). The oil was chromatographed on Sephadex LH-20, with 1:1 dichloromethane–methanol as eluant, to obtain three major fractions. The first fraction was discarded while the third fraction was chromatographed on silica gel. The fraction eluted with 1% 2-propanol in ether gave crystals of sednolide (4, 10 mg, 0.032 mg/animal) from ether. The middle fraction from Sephadex LH-20 chromatography was chromatographed on TLC-grade silica gel. Material eluted with dichloromethane was further fractionated by HPLC on Partisil, with ether as eluant, to obtain sednolide 23-acetate (5, 1 mg, 0.003 mg/animal) and 12-deacetyl-20-methyl-12-epideoxoscalarin (1, 33 mg, 0.1 mg/animal). Material eluted with 25% 2-propanol in dichloromethane was further purified by HPLC on Partisil with 2% 2-propanol in ether as eluant, to obtain 23-hydroxy-20-methyldeoxoscalarin (2, 2 mg, 0.006 mg/animal) and 23-hydroxy-20-methylscalarolide (3, 11 mg, 0.036 mg/animal).

12-Deacetyl-20-methyl-12-epideoxoscalarin (1): glass; $[\alpha]^{23}_D$ + 4.2° (*c* 0.33, $CHCl_3$); IR (KBr) 3420 (br), 1450, 1385, 1075 cm^{-1}; ^1H NMR (CCl_4) δ 0.75 (s, 3 H), 0.81 (s, 3 H), 0.84 (s, 3 H), 0.86 (s, 3 H), 0.92 (s, 3 H), 1.18 (d, 3 H, *J* = 7 Hz), 2.20 (m, 1 H), 3.12 (br s, 1 H, OH), 3.40 (dd, 1 H, *J* = 11, 5Hz), 3.64 (br s, 1 H, OH), 4.55 (br q, 1 H, *J* = 7 Hz), 5.28 (d, 1 H, *J* = 6 Hz), 5.34 (br s, 1 H, $w_{1/2}$ = 10 Hz); ^{13}C NMR ($CDCl_3$) see Table I; high-resolution mass spectrum, obsd *m/z* 384.3038, $C_{26}H_{40}O_2$ (M – H_2O) requires 384.3028.

23-Hydroxy-20-methyldeoxoscalarin (2): oil; IR (film) 3560, 1730, 1710, 1240, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.77 (s, 3 H), 0.79 (s, 3 H), 0.88 (s, 3 H), 1.10 (s, 3 H), 1.26 (d, 3 H, *J* = 6 Hz), 2.09 (s, 3 H), 2.82 (m, 1 H), 3.90 (d, 1 H, *J* = 13 Hz), 4.03 (d, 1 H, *J* = 13 Hz), 4.70 (br q, 1 H, *J* = 6 Hz), 4.89 (br s, 1 H, $w_{1/2}$ = 4 Hz), 5.21 (br s, 1 H), 5.34 (m, 1 H); mass spectrum, *m/z* 442 (M – H_2O).

23-Hydroxy-20-methylscalarolide (3): mp. 278–9 °C; UV (MeOH) 217 nm (ε 4725); IR (KBr) 3410, 3315, 1730, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.78 (s, 3 H), 0.86 (s, 3 H), 1.06 (s, 3 H), 1.14 (s, 3 H), 1.41 (d, 3 H, *J* = 6 Hz), 3.50 (dd, 1 H, *J* = 10, 6 Hz), 3.86 (d, 1 H, *J* = 12 Hz), 4.02 (d, 1 H, *J* = 12 Hz), 4.87 (q, 1 H, *J* = 6 Hz), 6.08 (s, OH); ^{13}C NMR ($CDCl_3$) δ 175.0, 166.0, 135.4, 79.1, 62.6, 58.5, 57.1, 55.4, 42.3, 41.8, 37.3, 34.4, 33.0, 29.7, 28.8, 24.5, 21.8, 18.5, 18.1, 16.7, 16.6 (only 22 of 26 signals observed); high-resolution mass spectrum, obsd, *m/z* 416.2939, $C_{26}H_{40}O_4$ requires 416.2927.

Sednolide (4): mp 268–272 °C; IR (KBr) 3400, 1755, 1455, 1385 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.77 (s, 3 H), 0.88 (s, 3 H), 0.89 (s, 3 H), 1.14 (s, 3 H), 1.35 (d, 3 H, *J* = 7 Hz), 2.30 (m, 1 H), 2.91 (br s, 1 H), 3.50 (br s, OH), 3.79 (dd, 1 H, *J* = 6 Hz), 3.96 (d, 1 H, *J* = 12 Hz), 4.50 (d, 1 H, *J* = 12 Hz), 4.45 (br q, 1 H, *J* = 7 Hz), 5.52 (m, 1 H, *J* = 3, 2, 2 Hz); high-resolution mass spectrum, obsd *m/z* 416.2938, $C_{26}H_{40}O_4$ requires 416.2927.

Sednolide 23-acetate (5): oil; ^1H NMR ($CDCl_3$) δ 0.86 (s, 2 H), 0.90 (s, 3 H), 0.91 (s, 3 H), 1.03 (s, 3 H), 1.37 (d, 3 H, *J* = 7 Hz), 2.10 (s, 3 H), 2.92 (br s, 1 H), 3.53 (br d, 1 H, *J* = 6 Hz), 3.84 (dd, 1 H, *J* = 10, 4 Hz), 4.19 (d, 1 H, *J* = 12 Hz), 4.46 (br q, 1 H, *J* = 7 Hz), 4.67 (d, 1 H, *J* = 12 Hz), 5.36 (br s, 1 H).

Dehydration of 12-deacetyl-20-methyldeoxoscalarin (1): A solution of the diol 1 (5 mg) and acetic anydride (0.5 mL) in pyridine (1 mL) was stirred at room temperature for 16 h. The solvents were distilled under high vacuum to obtain a residue that was purified by HPLC on Partisil, with 20% acetone in ether as eluant, to obtain the furan 8 (2 mg) as the major product: ^1H NMR ($CDCl_3$) δ 0.81 (s, 3 H), 0.84 (s, 6 H), 0.88 (s, 3 H), 1.18 (s, 3 H), 2.13 (s, 3 H), 2.60 (dd, 1 H, *J* = 16, 6 Hz, 3.2 (dd, 1 H, *J* = 12.5, 4 Hz), 7.40 (s, 1 H).

Single-Crystal X-ray diffraction analysis of sednolide (4): Preliminary X-ray photographs of a suitable crystal of sednolide (4) showed orthorhombic symmetry. Lattice parameters of *a* = 8.328 (4), *b* = 14.419 (6), and *c* = 19.541 (6) Å were determined from a least-squares fit of 15 diffractometer-measured 2θ values. Systematic absences, the presence of chirality, and density considerations (ρ_c = 1.18 g/cm^3 for *Z* = 4) uniquely indicated space group $P2_12_12_1$ with one molecular of composition $C_{26}H_{40}O_4$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a computer-controlled four-circle diffractometer using graphite-monochromated Cu Kα (1.54178 Å) X-rays and variable speed, 1° ω scans. Of the 1917 noncheck reflections collected, 1541 (85%) were judged observed after correcting for Lorentz, polarization, and background effects ($|F_0| \geq 3\sigma|F_0|$). The structure was determined by using a multisolution tangent formula approach.[8] An *E* synthesis of the most favorable set revealed 28 of the 30 non-hydrogen atoms. After partial refinement, a ($2F_0 - F_0$) synthesis revealed the entire molecule. A difference electron density synthesis revealed most of the hydrogens, and the remainder were included at chemically reasonable positions. Block-diagonal least-squares refinements with anisotropic non-hydrogen atoms and isotropic hydrogens have converged to a standard crystallographic residual of 0.070 for the observed reflections. Bond distances and angles agree well with generally expected values, and the paragraph at the end of this paper on supplementary material describes additional crystallographic material.

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Registry No. 1, 85337-12-8; 2, 85354-71-8; 3, 85337-13-9; 4, 85337-14-0; 5, 85337-15-1; 8, 85337-16-2.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, bond distances, and bond angles (5 pages). Ordering information is given on any current masthead page.

(8) All crystallographic calculations were done on a PRIME 400 computer operated by the Materials Science Center and the Department of Chemistry, Cornell University. The principal programs used were REDUCE and UNIQUE, data reduction programs [Leonowicz, M. E., Cornell University, 1978]; BLS78A, anisotropic block-diagnoal least-squares refinement [Hirotsu, K.; Arnold, E., Cornell University, 1980]; XRAY76, [The X-ray System of Crystallographic Programs, Stewart, J. M., Ed., University of Maryland, Technical Report TR-445, March 1976]; ORTEP, crystallographic illustration program [Johnson, C. K., Oak Ridge, TN, ORNL-3794]; BOND, molecular metrics program, [Hirostu, K., Cornell University, 1978]; MULTAN-78 ["A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data", University of New York, England], principal author, P. Main. For literature description of MULTAN, see: Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr., Sect. B* **1970**, *26*, 274–285. Woolfson, M. M. *Acta Crystallogr., Sect. A.* **1977**, *33*, 219–225.