STRUCTURES OF TETRA-O-DEMETHYLCOLCHICINE, -ISOCOLCHICINE, AND 10-O-DEMETHYLCOLCHICINE DERIVATIVES[1]

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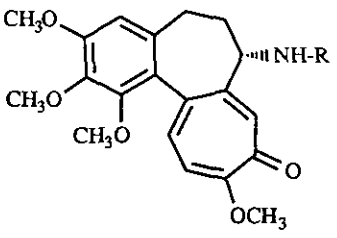
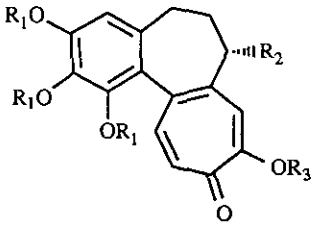
Abstract – During the exhaustive demethylation of both colchicine and isocolchicine analogs, tetra-O-demethyl derivatives with identical structures were produced. Spectral examination (^{1}H-^{13}C long-range COSY nuclear magnetic resonance) of these analogs (**5** – **8**) indicated that isocolchicine-type tautomerism is predominant in tetra-O-demethylcolchicine derivatives. Similarly, structures of 10-O-demethylcolchicine derivatives were revised to be an isocolchicine-type shown by formulae (**9**) and (**10**).

Colchicine (**1**), a major alkaloid presented in Colchicum autumnale, has been extensively investigated, and the principal biological action of this drug as a microtubule spindle toxin is well established.[2] Recently, we prepared the tetrademethyl derivatives of N-trifluoroacetyldeacetylcolchicine (**2**) and N-(3',4',5'-trihydroxybenzoyl)-deacetylcolchicine (the structures were formerly proposed as **11** and **12**, respectively), and found that these cytocidal analogs exhibited interesting biological activities not shared by **1**, including the inhibition of DNA unknotting by purified mammalian DNA topoisomerase II.[3] In order to further examine this series of analogs as

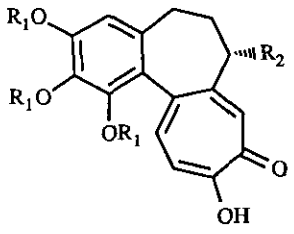
inhibitors of DNA topoisomerase II, additional tetra-O-demethyl compounds, including tetra-O-demethylisocolchicine derivatives, were prepared. During their preparation, it was found that both colchicine and isocolchicine compounds afforded identical tetra-O-demethyl derivatives with isocolchicine-type tautomerism. This finding prompted our re-examination of the tautomerism of the tropolone ring in both tetra-O-demethylcolchicine and 10-O-demethylcolchicine analogs. We report here on the characterization of tetra-O-demethylcolchicine, -isocolchicine, and 10-O-demethylcolchicine analogs.



1 R=$COCH_3$
2 R=$COCF_3$



	R_1	R_2	R_3
3	CH_3	$NHCOCH_3$	CH_3
4	CH_3	$NHCOCF_3$	CH_3
5	H	$NHCOCF_3$	H
6	H	NHCO–	H
7	H	$NHCOCH_3$	H
8	H	NH_2•HBr	H
9	CH_3	NH_2	H
10	CH_3	$NHCOCF_3$	H

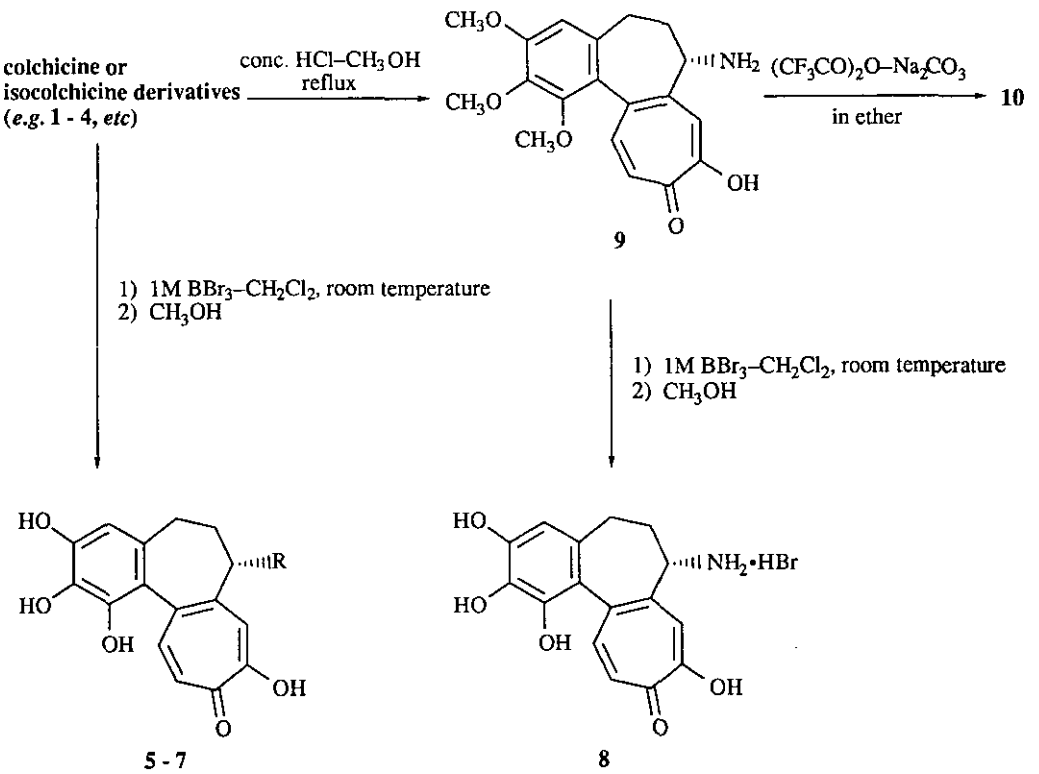


	R_1	R_2
11	H	$NHCOCF_3$
12	H	NHCO–
13	CH_3	NH_2
14	CH_3	$NHCOCH_3$

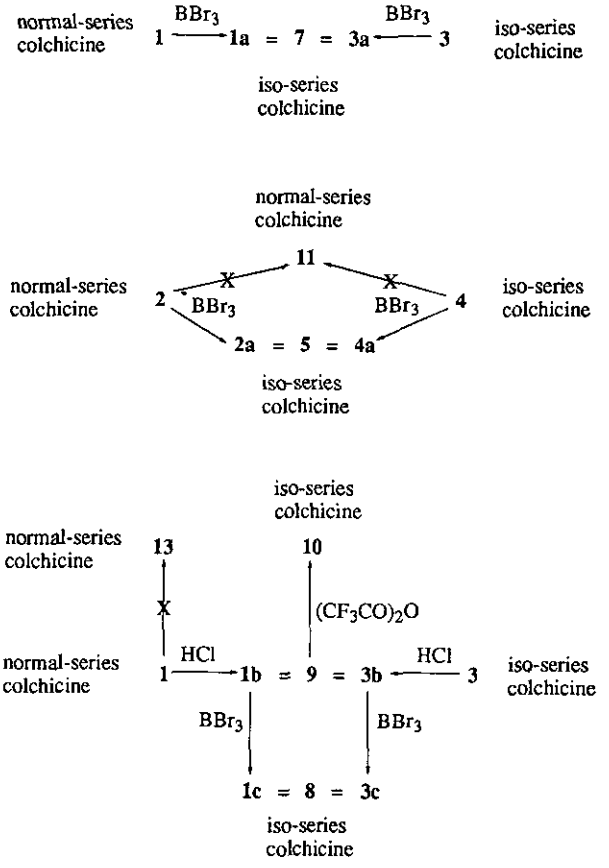
Figure 1

The general procedures used for preparing tetra-O-demethylcolchicine and -isocolchicine analogs are outlined in Schemes I and II. Demethylation of colchicine (**1**) and its trifluoroacetyl derivative (**2**) with boron tribromide[7] afforded products designated **1a** and **2a**, while treatment of isocolchicine (**3**) and its trifluroacetyl derivative (**4**) as for **1** furnished tetra-O-demethyl products designated **3a** and **4a**. The ^1H and ^{13}C nmr spectra of **1a** and **3a** were identical, as were those of **2a** and **4a**, indicating that either tautomerism of the colchicine-type or isocolchicine-type is predominant in these tetrademethyl analogs (these compounds were later identified as structures (**7**) and (**5**),

respectively). Before further spectral examination of the trifluoro derivative (**5**), the ^{1}H-^{13}C long-range COSY spectra of **1** and **4** were taken to determine the differences in the long-range correlations between the normal- (**1**) and iso-series (**4**) colchicines.



Scheme I. Syntheses of 1,2,3,10-O-Tetrademethylisocolchicine (**5 – 8**) and 9-O-Demethylisocolchicine Derivatives (**9, 10**).



Scheme II. Reactions of Normal- and Iso-Series Colchicines

The ^{1}H-^{13}C long-range correlations in **1** and **4** are shown in Figures 2 and 3. In the ^{13}C nmr of **1**, the carbon resonances at δ 179.4 and 164.9 were assignable to C-9 and C-10, respectively, while the ^{13}C nmr of **4** showed the resonances due to C-9 and C-10 at δ 164.8 and 179.3, respectively. In **1**, the carbonyl resonance at C-9 (δ 179.4) exhibited a long-range correlation with H-11 at δ 6.98 through a three-bond coupling. The resonance for C-10 (δ 164.9) revealed long-range coupling with H-8 (δ 7.30) and H-12 (δ 7.17). In contrast, in the ^{1}H-^{13}C long-range COSY of **4**, correlations between the carbonyl resonance at C-10 (δ 179.3) and H-8 (δ 7.15) and -12 (δ 7.34) were observed, while the resonance at δ 164.8 (C-9) exhibited long-range coupling with H-11 (δ 7.02). Since differences were observed in the long-range correlations of the tropolone ring for colchicine- and

isocolchicine-type structures, examination of the ^{1}H-^{13}C long-range COSY of **5** was carried out. The long-range correlations in **5** are summarized in Figure 4.

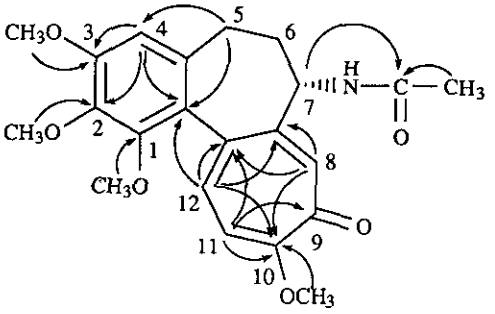


Figure 2. ^{1}H-^{13}C Long-range Correlations in **1**
[Acetone-d_6 (J_{C-H} = 10 Hz)]

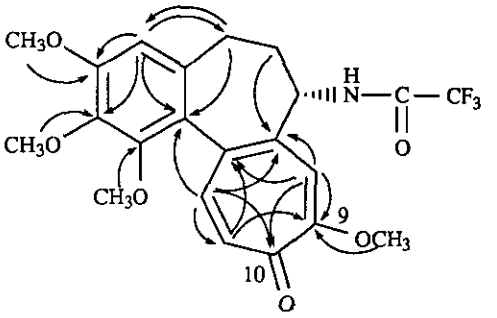


Figure 3. ^{1}H-^{13}C Long-range Correlations in **4**
[Acetone-d_6 (J_{C-H} = 10 Hz)]

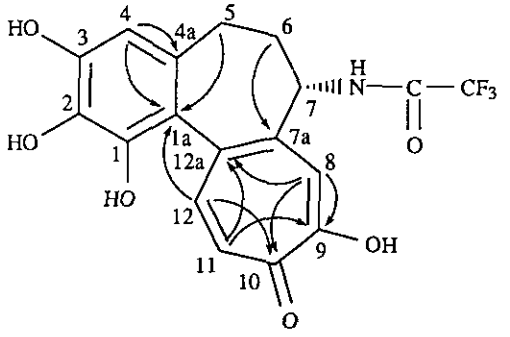


Figure 4. ^{1}H-^{13}C Long-range Correlations in **5**
[Acetone-d_6 (J_{C-H} = 10 Hz)]

The carbon resonance at δ 173.0, assignable to the carbonyl carbon on the tropolone ring, exhibited long-range correlations with a singlet at δ 7.48 and with a doublet at δ 7.59. The former proton signal was easily ascribed to H-8, while the latter one could be assigned to either H-11 or H-12. This proton signal at δ 7.59 also showed a correlation with a quaternary carbon signal at δ 119.3. Since this carbon resonance was further coupled with H_2-5 (δ ca. 2.31 and 2.45) and H-4 (δ 5.42) through a three-bond coupling in each case, it was assigned to C-1a. Therefore, the proton signal at δ 7.59 was established as H-12. The long-range correlations in **5** are the same as

those found in **4**; thus, the carbonyl group in **5** should be located at C-10. Similar spectral examination of the other tetra-O-demethyl analogs (**6** and **7**) also indicated that the carbonyl group was at C-10. Based on these spectral examinations, the structures of the tetra-O-demethylcolchicine and -isocolchicine analogs are both of the isocolchicine-type and should be represented by formulae (**5** – **7**). Previously, we reported the structures of compounds (**5**) and (**6**) to be **11** and **12**, respectively;[3] thus, these structures should be revised. The complete assignments of carbon resonances for these derivatives were established by the examination described above and are shown in Table 1.

This revision of colchicine- to isocolchicine-type structures in these tetra-O-demethyl compounds prompted us to re-examine proposed structures of 10-O-demethylcolchicine derivatives (the structures were formerly proposed as **13** and **14**). Hydrolysis of colchicine or isocolchicine (**1** or **3**) with concentrated hydrochloric acid in methanol afforded 10- or 9-O-demethyldeacetyl products designated as **1b** and **3b**,[6] respectively, which were subsequently demethylated with boron tribromide to give the tetra-O-demethyldeacetyl derivatives (Schemes I and II). These latter derivatives were identical (compound **8**) and were of the isocolchicine-type. Also, the ^{1}H and ^{13}C nmr spectra of **1b** and **3b** were identical (compound **9**). The hydrolyzed product from **1** was formerly considered to be 10-O-demethyldeacetylcolchicine (or trimethylcolchicinic acid, structure **13**).[4,5] The carbon resonances arising from the tropolone ring of trimethylcolchicinic acid (**13**) were similar to those of colchicine (**1**), as described in the literature.[4,5] Thus, the proposed structure seemed reasonable. However, the ^{1}H-^{13}C long-range COSY of **9** exhibited similar correlations to those found in **5**. Especially, the correlations between the carbonyl resonance at δ 174.1 and H-8 (δ 6.81) and H-12 (δ 7.35) indicated that the carbonyl group should be placed at C-10. Accordingly, the structure of this demethyldeacetylcolchicine should be of the isocolchicine-type and be represented by formula (**9**).

Compound (**10**) was obtained by partial trifluoroacetylation of **9** with trifluoroacetic anhydride and anhydrous sodium carbonate in ether. As for compounds (**5** – **9**), the presence of an isocolchicine-type tautomerism was confirmed again by ^{1}H-^{13}C long-range COSY examination; therefore, the correct structure for this product is **10**, instead of **14**.

These findings described above suggest that all tetra-O-demethylcolchicine as well as the tropolone ring mono-O-demethylcolchicine derivatives, possess an isocolchicine-type structure.

Table 1. ^{13}C Nmr Data for **1**, **4**, **5** – **10** (75.5 MHz)

	1[a]	4[a]	5[a]	6[a]	7[a]	8[b]	9[a]	10[a]
1	152.0	151.8	149.0	151.9	151.6	147.1	151.3	151.6
2	142.5	142.6	143.9	143.5	143.5	143.6	142.1	142.5
3	154.4	155.0	146.8	146.6	146.6	145.1	154.6	154.9
4	108.5	108.9	107.9	107.9	107.9	108.0	108.3	108.8
5	30.4	29.8	29.8	30.3	30.3	29.2	30.9	30.3
6	37.1	38.0	38.4	38.7	39.0	37.6	40.7	37.4
7	52.7	54.2	53.8	53.7	53.1	54.4	62.0	54.0
8	131.5	109.7	117.7	118.5	118.5	119.7	119.2	117.2
9	179.4	164.8	168.8	168.6	168.7	170.3	167.8	169.0
10	164.9	179.3	173.0	172.9	172.8	171.5	174.1	173.2
11	112.5	134.8	124.9	124.7	124.7	124.3	125.4	125.0
12	135.1	141.1	143.9	142.3	143.5	142.3	142.3	142.1
13	169.3	157.2	157.3	–	170.1	–	–	157.5
14	22.7	117.0	116.8	–	22.8	–	–	117.0
1a	126.9	126.6	119.3	120.1	120.0	118.4	126.6	126.6
4a	135.3	135.8	132.9	133.3	133.2	132.8	136.6	135.4
7a	151.7	142.9	131.1	131.3	131.2	130.3	152.3	148.8
12a	136.5	134.8	137.3	137.2	137.5	137.9	136.3	136.0
Galloyl								
C-1				126.1				
C-2				107.9				
C-3				146.1				
C-4				137.4				
–CO				167.0				
MeO-C(1)	61.4	61.4					61.2	61.3
MeO-C(2)	61.2	61.2					61.1	61.2
MeO-C(3)	56.4	56.2					56.3	54.0
MeO-C(9 or 10)	56.4	56.4					–	–

a : Measured in Acetone-d_6
b : Measured in Acetone-d_6 + D_2O

EXPERIMENTAL

Optical rotations were determined using a Rudolph Research Autopol III polarimeter. Ir spectra were recorded on a Perkin Elmer 1320 infrared spectrophotometer. ^{1}H and ^{13}C nmr spectra were recorded on a Bruker AC-300 (300 and 75.5 MHz, respectively) spectrometer. Chemical shifts are presented in terms of δ (ppm) with tetramethylsilane as an internal standard. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Toyopearl (HW-40F) from TOSOH Corp., (Tokyo, Japan) was used for column chromatography. Colchicine (**1**) was purchased from Aldrich, Inc. (Milwaukee, WI).

General Procedures for Exhaustive Demethylation of 1 Analogs. To a solution of a colchicine or isocolchicine analog (0.1 – 1 mmol) in anhydrous CH_2Cl_2 (5 – 15 ml) was added dropwise a 1M solution of boron tribromide in CH_2Cl_2 (0.3 – 4.0 mmol) under ice cooling. The reaction mixture was maintained at 0 °C for 1 h and then stirred at room temperature overnight. The reaction mixture was cooled using an ice bath, and methanol was then added dropwise over 1 h . The solvent was evaporated under reduced pressure, and the residue was purified by Toyopearl HW-40F column chromatography using water and then methanol as eluents.

1,2,3,9-Tetra-O-demethyl-N-trifluoroacetyldeacetylisocolchicine (5). Yield 75%. A tan amorphous powder. $[\alpha]_D^{20}$ –304° (c=0.11, EtOH). Anal. Calcd for $C_{18}H_{14}NO_6F_3$ $2H_2O$: C, 49.89; H, 4.19; N, 3.23. Found: C, 49.95; H, 3.98; N, 3.11. ^{1}H Nmr (Acetone-d_6, 300 MHz): δ ca. 2.29 (m, 2H, H-6), ca. 2.31, 2.45 (each 1H, m, H-5), 4.77 (1H, dd, J = 6, 11 Hz, H-7), 6.42 (s, 1H, H-4), 7.36 (1H, d, J = 12 Hz, H-11), 7.56 (1H, s, H-8), 7.78 (1H, d, J = 12 Hz, H-12). ^{13}C Nmr; see Table 1.

1,2,3,9-Tetra-O-demethyl-N-(3',4',5'-trihydroxybenzoyl)deacetylisocolchicine (6). Yield 34%. A tan amorphous powder. $[\alpha]_D^{20}$ –153° (c=0.10, EtOH). Anal. Calcd for $C_{23}H_{19}NO_9$ $3/2H_2O$: C, 57.50; H, 4.62; N, 2.92. Found: C, 57.07; H, 4.43; N, 2.61. Ir υmax: 3260 (OH) and 1600 (CO) cm^{-1}. ^{1}H Nmr (Acetone-d_6, 300 MHz): δ ca. 2.28 (2H, m, H-6), ca. 2.25, 2.46 (each 1H, m, H-5), 4.85 (1H, m, H-7), 6.41 (1H, s, H-4), 7.04 (2H, s, H-2',6'), 7.25 (1H, d, J = 12 Hz, H-11), 7.61 (1H, s, H-8), 7.68 (1H, d, J = 12 Hz, H-12), 8.15 (1H, d, J = 6.83 Hz, HN-7). ^{13}C Nmr; see Table 1.

1,2,3,9-Tetra-O-demethylisocolchicine (7). Yield 71%. A tan amorphous powder. $[\alpha]_D^{20}$ –357° (c=0.2, EtOH). Anal. Calcd for $C_{18}H_{17}NO_6$ $5/4H_2O$: C, 59.09; H, 5.37; N, 3.83. Found: C, 59.18; H, 5.19; N, 3.77. Ir υmax: 3340, 3320, and 3070 (OH and NH), 2930 and 2850 (aliphatic C-H), 1640 (C=O, amide), and 1620

(C=O, tropolone) cm^{-1}. ^1H Nmr (Acetone-d6, 300 MHz): δ ca. 2.19 (2H, m, H-6), ca. 2.25, 2.42 (each 1H, m, H-5), 4.61 (1H, m, H-7), 6.41 (1H, s, H-4), 7.37 (1H, d, J = 12 Hz, H-11), 7.66 (1H, s, H-8), 7.75 (1H, d, J = 12 Hz, H-12), 8.31 (1H, d, J = 7 Hz, NHCOCH3). ^{13}C Nmr; see Table 1.

9-O-Demethyldeacetylisocolchicine (Trimethylcolchinic acid) (9). Trimethylcolchicinic acid (**9**) was prepared according to the procedure described in the literature.[6] To a solution of colchicine (**1**) (5g, 12.5 mmol) in methanol (30 ml) was added conc. HCl (30 ml), and the mixture was refluxed at 90 °C for 24 h. After cooling, the mixture was neutralized with 5% $NaHCO_3$ solution and extracted with CH_2Cl_2 (600 ml) three times. The CH_2Cl_2-layer was washed with brine (300 ml), dried over Na_2SO_4, and concentrated under reduced pressure to give a crude product. Crystallization with CH_2Cl_2-CH_3OH afforded **9** (2.2 g). Isocolchicine (**3**) (500 mg, 0.13 mmol) was also treated with conc. HCl in the same manner as **1** to yield **9** (190 mg). The physical data of **9** was identical with those described in the literature.[6]

9-O-Demethyl-N-triflioroacetyldeacetylisocolchicine (10). Compound **10** was prepared according to the procedure described in the literature.[8] To a suspension of **9** (97 mg, 0.29 mmol) and Na_2CO_3 (310 mg, 2.9 mmol) in ether (8 ml), trifluoroacetic anhydride (0.4 ml, 2.9 mmol) was added under ice-cooling, and the mixture was further stirred at room temperature for 3 h. After removal of the inorganic salts by filtration, the filtrate was extracted with CH_2Cl_2 (50 ml). The organic layer was washed with 5% $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated to give a crude product. Crystallization from benzene-CH_2Cl_2 yielded **10** (90 mg). The physical and spectral data was identical with those described in the literature.[8]

1,2,3,9-Tetra-O-demethyldeacetylisocolchicine Bromide (8). To a solution of **9** (300 mg, 0.87 mmol) in CH_2Cl_2 (7 ml) was added dropwise a 1M solution of boron tribromide (2.8 mmol) under ice cooling. The mixture was maintained at 0 °C for 1 h and then stirred at room temperature overnight. The reaction mixture was worked up as described in the general procedure to furnish **8** (102 mg). Yield 30%. A tan amorphous powder. $[\alpha]_D^{20}$ –265° (c=0.12, EtOH). Anal. Calcd for $C_{16}H_{15}NO_5$ HBr H_2O: C, 48.02; H, 4.53; N, 3.50; Br, 19.96. Found: C, 48.38; H, 4.48; N, 3.47; Br, 20.10. Ir υmax: 3350, 3200, and 3050 (OH and NH), 2920 and 2860 (aliphatic C-H) and 1600 (C=O, tropolone) cm^{-1}. ^1H Nmr (Acetone-d6 + D_2O, 300 MHz) δ 2.25 - 2.70 (4H, m, H-5, 6), 4.29 (1H, m, H-7), 6.45 (1H, s, H-4), 7.34 (1H, d, J = 12 Hz, H-11), 7.65 (1H, s, H-8), 7.72 (1H, d, J = 12 Hz, H-12). ^{13}C Nmr; see Table 1.

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REFERENCES

1. Antitumor Agents 142. For part 141, see L. Sun, E. Hamel, C. M. Lin, S. B. Hastie, A. Pyluck, and K. H. Lee, J. Med. Chem., in press.
2. a) A. Brossi, J. Med. Chem. 1990, **33**, 2311. b) S. B. Hastie, Pharmac. Ther. 1991, **51**, 377.
3. K. F. Bastow, H. Tatematsu, I. D. Bori, Y. Fukushima, L. Sun, B. Goz, and K. H. Lee, J. Bioorg. & Med. Chem. Lett., 1993, **3**, 1045.
4. C. D. Hufford, H. G. Capraro, and A. Brossi, Helvetica Chim. Acta, 1980, **63**, 50.
5. C. D. Hufford, C. C. Collins, and A. M. Clark, J. Pharm. Sci., 1979, **68**, 1239.
6. Von D. H. Fernholz, Angew. Chem., 1953, **65**, 319.
7. E. H. Vickery, L. F. Pahler, and E. J. Eisenbraun, J. Org. Chem., 1979, **44**, 4444.
8. H. G. Capraro and A. Brossi, Helvetica Chim. Acta, 1979, **62**, 965.

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