Antitumor Agents

Part 184[1])

Syntheses and Antitubulin Activity of Compounds Derived from Reaction of Thiocolchicone with Amines: Lactams, Alcohols, and Ester Analogs of Allothiocolchicinoids

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Dedicated to Prof. *Dieter Seebach* on the occasion of his 60th birthday

7-*O*-Substituted analogs of deaminodeoxycolchinol thiomethyl ether were synthesized and evaluated for their inhibitory effects on tubulin polymerization *in vitro*. Ketone **9**, a key compound in this study, was derived from thiocolchicone **6** by reaction with aniline. Reaction of compound **6** with $MeNH_2$ or $BuNH_2$ gave tetracyclic lactams **7** and **8**, respectively. Optically active alcohols **11a** and **11b** were obtained from racemic **11** by chemical resolution including a separation of the camphanate diastereoisomers **12a** and **12b**, followed by basic hydrolysis. The (a*R*,7*R*)-configuration of **12b** was verified by X-ray crystallographic analysis. Almost all racemic and optically active 7-*O*-acyl or 7-*O*-aroyl compounds had strong inhibitory effects on the tubulin polymerization reaction, with IC_{50} values from 1.7 to 5.1 μM. A few agents, such as the lactams **7** and **8**, the camphanates **12a** and **12b**, the cyclohexanecarboxylates **19a** and **19b**, and, most notably, the (7*S*)-benzoate **15a**, had negligible effects on polymerization, yielding IC_{50} values greater than 40 μM. Ketone **9** showed strong inhibition of tubulin polymerization comparable to that of thiocolchicone (**6**). Optically active alcohol **11a** and acyl esters **13a** and **14a** with a (7*S*)-configuration were more active than the (7*R*)-esters **13b** and **14b**. However, the esters **15a**–**17a** with a (7*S*)-configuration were less active than the (7*R*)-isomers **15b**–**17b**, in which the (7*R*)-benzoate **15b** was at least 15-fold more inhibitory than the (7*S*)-isomer **15a**. For the most part, the agents causing strongest inhibition of polymerization also caused the greatest inhibition of [^{3}H]colchicine binding. NMR and optical rotatory data indicate that, in polar solvents, the equilibrium in esters with a 7-*O*-aroyl substituent, *i.e.*, **15a**,**b**, **16a**,**b**, and **17a**,**b**, is reversed from (a*S*) to (a*R*) or from (a*R*) to (a*S*), as compared to nonpolar solvents.

[1]) Part 183: [1].

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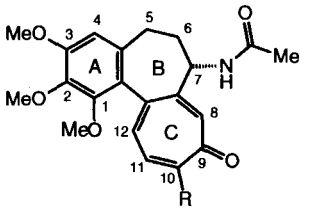
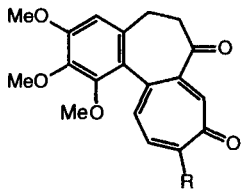
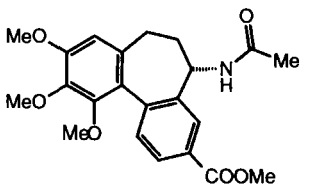
Introduction. – Colchicine (**1a**), an alkaloid from *Colchicum autumnale* and *Gloriosa superba*, is used to treat gout and familial Mediterranean fever [2]. Neither colchicine (**1a**) nor thiocolchicine (**2a**), the thiomethyl-ether analog of **1a** [3], has found a role as an anticancer agent, because of their toxicity.



1a R = OMe
2a R = SMe



5 R = OMe
6 R = SMe



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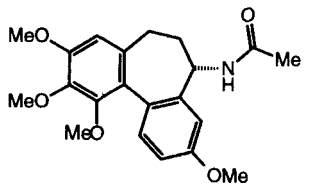

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Nevertheless, the interactions of colchicine (**1a**) and its analogs with its molecular target tubulin have been extensively studied. Among the important observations are findings indicating that the conformation and configuration of colchicinoids are important for binding to tubulin. Natural colchicine and potent analogs all have a (−)-(a*S*,7*S*)-configuration, while the (+)-(a*R*,7*R*)-antipodal isomers bind poorly to tubulin [4][5]. This conclusion regarding the stereoselectivity of the tubulin-colchicinoid interaction was further supported by our recent studies on (+)-thiocolchicine [6] and *O*-substituted thiocolchicinol analogs [7]. The (−)-(a*S*,7*S*)-isomers, as in previous work with other compounds, exhibited more potent antitubulin activity than the (+)-(a*R*,7*R*)-isomers, and the former were also more potent as inhibitors of *Burkitt* lymphoma cell growth.

Allocolchicine (**3**) and *N*-acetylcolchicinol *O*-methyl ether (**4**) [8][9] are two members of the allo series of colchicine analogs with a benzenoid *C* ring rather than a tropolonic *C* ring, as occurs in colchicine. The (7*S*)-allocolchicinoids, like (7*S*)-colchicinoids, energetically prefer an (a*S*) biaryl configuration, and these analogs also bind strongly to tubulin with activity comparable to that of colchicine.

We recently reported that thiocolchicone (**6**), the thiomethyl-ether analog of naturally occurring colchicone (**5**) [8], and selected analogs are potent inhibitors of tubulin polymerization [7] and of the growth of a human cancer cell line [10]. Furthermore, the elegant work of *Banwell et al.* suggested that allocolchicinoids should be accessible by

total synthesis [11]. These findings led us to prepare ketone **9** as a precursor to allothiocolchicinoids. We also believed that the nucleophilic reaction of thiocolchicone (**6**) with amines would result in the formation of allo-congeners with a benzenoid *C* ring, since a comparable reaction occurs when thiocolchicine (**2a**) is treated with MeSNa in MeOH [12].

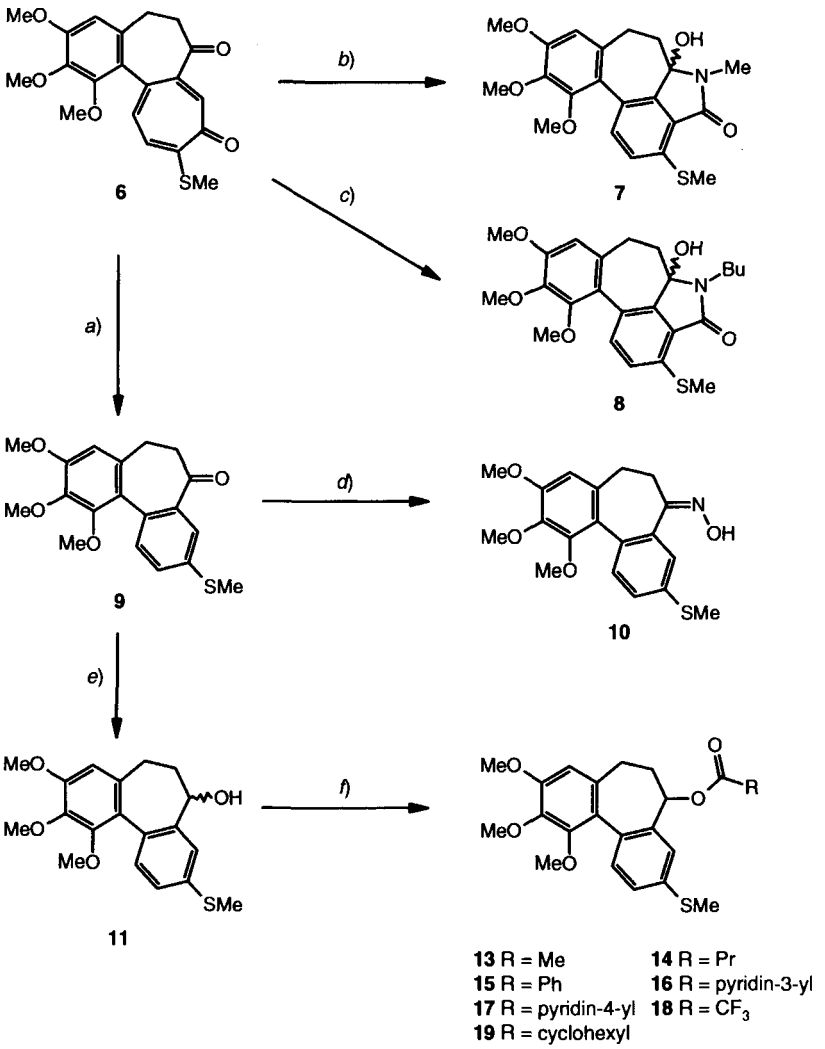
Thus, in this study, we report the synthesis, chemical characterization, and biological evaluation of a series of novel allothiocolchicone derivatives, including the racemic compounds **10**–**19**, including a diastereoisomer mixture **12**, and the antipodal isomers **11a**–**19a** and **11b**–**19b** (except **12a** and **12b**, which are diastereoisomers). Unexpectedly, introduction of *O*-aroyl substituents at C(7) of the new compounds resulted in different effects on the conformation of the biphenyl backbone and, therefore, on biological activity. The biologically active *O*-acyl derivatives retained the (−)-(a*S*,7*S*)-configuration of colchicine, but, among the *O*-aroyl derivatives, the (+)-(7*R*)-isomers had greater inhibitory effects on tubulin than the (−)-(7*S*)-derivatives. Detailed analysis of the ^{1}H-NMR spectra and optical rotatory data indicated that, in solution, both (7*S*)- and (7*R*)-compounds assumed two conformations, and that biological activity was related to the proportion of the (a*S*)-conformation.

Chemistry. – Thiocolchicone (**6**) was prepared from deacetylthiocolchicine as described in [7]. When refluxed in toluene, reaction of **6** with $MeNH_2$ and $BuNH_2$ (*Scheme 1*) gave tetracyclic lactams **7** and **8**, whose structures were elucidated from spectral evidence. The structure of **7** was further confirmed by X-ray analysis (see *Exper. Part*). A view of the solid-state conformation of one enantiomer of **7** is illustrated in *Fig. 1*. Endocyclic torsion angles characterizing the conformation of ring *B* (*Fig. 1*) (ω_{ij}(°) about bonds between atoms *i* and *j*: $\omega_{4a,5}$ 75.8(2), $\omega_{5,6}$ −40.1(2), $\omega_{6,6a}$ −38.5(2), $\omega_{6a,12c}$ 64.7(3), $\omega_{12c,12a}$ 0.2(3), $\omega_{12a,12b}$ −39.6(3), $\omega_{12b,4a}$ −11.2(3)) are related by an approximate C_2 symmetry axis passing through C(6) and the mid-point of the C(12a)–C(12b) bond, and the ring has a distorted twist-boat conformation. The reaction of **6** with $PhNH_2$ took a different course and yielded the desired allo-ketone **9** in 39% yield. The reaction of **6** with $PhNH_2$ must be greatly assisted by the presence of a C(7)=O group since it did not occur with thiocolchicine or deacetylthiocolchicine [1].

Ketone **9**, like compound **6**, is a racemic mixture with its chiral biphenyl backbone in a (a*S*) ⇌ (a*R*) equilibrium. The structure of **9** was verified by UV, ^{1}H-, ^{13}C-, and 2D-NMR data [1]. The strong absorbance at 340 nm, which is caused by the tropolone moiety and observed in most colchicinoids, was not present in the allo-ketone **9** and its derivatives. Reduction of **9** with $NaBH_4$ gave alcohol **11**, which on esterification with various commercially available acid chlorides or with Ac_2O afforded the corresponding racemic esters **13**–**19**. Compound **10** was obtained by reacting **9** with $NH_2OH \cdot HCl$ in the presence of AcONa.

Reaction of **11** with optically pure (−)-(1*S*)-camphanic chloride (*Scheme 2*) yielded a mixture of camphanates **12a**/**12b**, which were separated by flash column chromatography on silica gel to afford the optically active esters **12a** and **12b**. Basic hydrolysis of **12a** and **12b** led to alcohols **11a** and **11b**, respectively; these enantiomers have identical melting points, NMR spectra, and TLC properties, but opposite optical rotations. Esterification with acid chlorides or acid anhydrides yielded optically active esters **13a**–**17a**, **19a**, and **13b**–**17b**, **19b**.

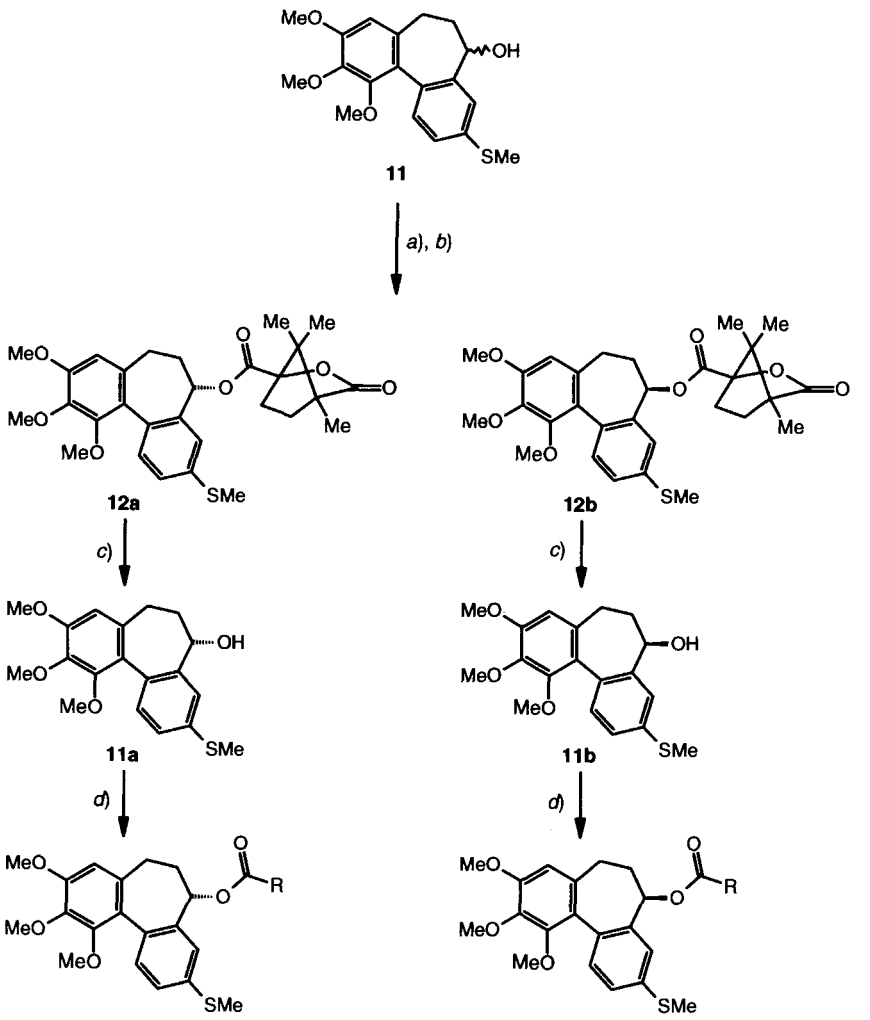
Scheme 1



a) $PhNH_2$/PhH. *b*) $MeNH_2$/PhH. *c*) $BuNH_2$/PhH. *d*) NH_2OH/AcONa/MeOH, CH_2Cl_2. *e*) $NaBH_4$/MeOH, CH_2Cl_2. *f*) RCOCl or $(RCO)_2O$/pyridine.

The complete structure and absolute configuration of **12b** with (a*R*,7*R*)-configuration were established unequivocally from NMR, UV, and MS data, elemental analysis, and X-ray crystallographic analysis (see *Exper. Part* and [1]). A view of the solid-state conformation is presented in *Fig. 2*. The overall absolute configuration was defined by that of the (1′*S*)-camphanoyl moiety. As in **7** above, endocyclic torsion angles in seven-membered ring *B* ($\omega_{4a,5}$ 71.6(4), $\omega_{5,6}$ −43.8(4), $\omega_{6,7}$ −45.0(4), $\omega_{7,7a}$ 77.5(4), $\omega_{7a,11a}$ −2.9(5), $\omega_{11a,11b}$ −52.1(4), $\omega_{11b,4a}$ 3.3(5)°) are related by an approximate C_2-symmetry axis passing through C(6) and the mid-point of the C(11a)–C(11b) bond; thus,

Scheme 2



13a R = Me **14a** R = Pr **13b** R = Me **14b** R = Pr
15a R = Ph **16a** R = pyridin-3-yl **15b** R = Ph **16b** R = pyridin-3-yl
17a R = pyridin-4-yl **19a** R = cyclohexyl **17b** R = pyridin-4-yl **19b** R = cyclohexyl

a) (−)-Camphanic chloride. *b*) Column chromatography. *c*) 2N NaOH. *d*) RCOCl or $(RCO)_2O$/pyridine.

the ring has a distorted twist-boat form with the substituent at C(7) in a pseudo-equatorial orientation. Furthermore, the C(4a)–C(11b)–C(11a)–C(7a) and C(11)–C(11a)–C(11b)–C(1) torsion angles of −52.1(4)° and −56.2(5)°, respectively, and the dihedral angle of 54.9° between the least-squares planes through the *A* and *C* ring atoms indicate that **12b** has an (a*R*)-biaryl configuration. The (a*R*,7*R*)-absolute configuration determined for **12b** appears to establish an equivalent configuration for the other

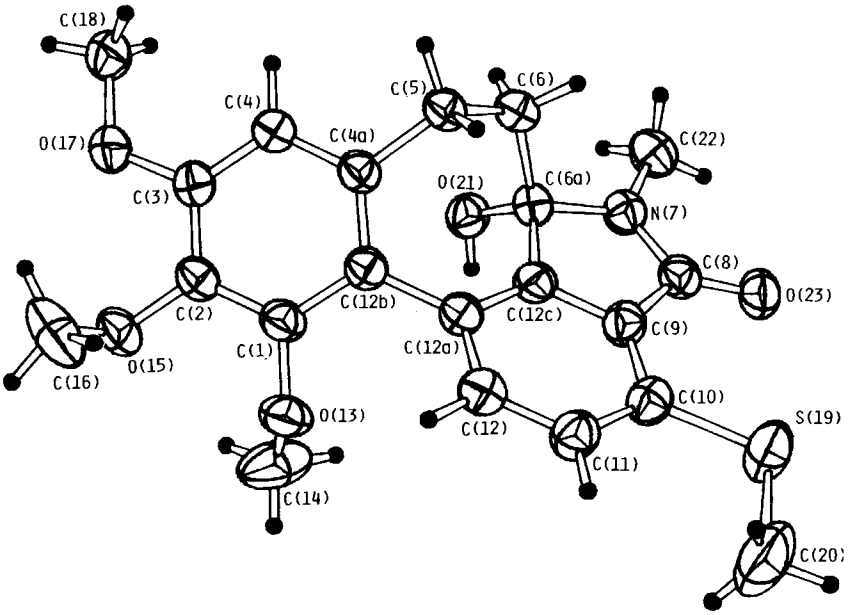


Fig. 1. *ORTEP Diagram* (50% probability ellipsoids) *showing the crystallographic atom-numbering scheme and solid-state conformation of one enantiomer in crystals of racemic alcohol* **7**. Small filled circles represent H-atoms.

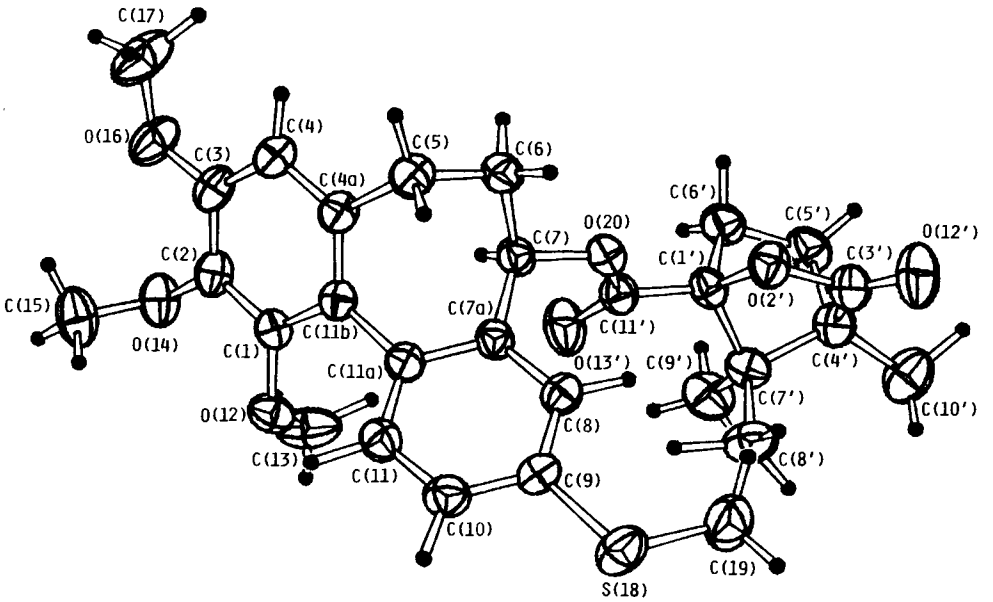


Fig. 2. *ORTEP Diagram* (40% probability ellipsoids) *showing the crystallographic atom-numbering scheme and solid-state conformation of* **12b**. Small filled circles represent H-atoms.

esters (**11b**, **13b**–**19b**) derived therefrom. Compounds **12a** and its derivatives, **11a** and **13a**–**19a**, should have an (a*S*,7*S*)-configuration.

However, detailed analysis of solutions of racemic and optically active allo-congeners, including the alcohols and their esters, demonstrated that these compounds are in a solvent-dependent conformational equilibrium [1]. In $CHCl_2$, (a*S*)-conformer is the major conformer for all (7*S*)-allo-congeners; (a*R*)-conformer is the minor conformer. As the polarity of the solvent increases, the (a*S*)/(a*R*) ratio decreases. For the (7*R*)-analogs, (a*R*)-conformer predominates in $CHCl_3$, but the (a*S*)/(a*R*) ratio increases as the solvent polarity increases [1]. This was not the case with the tropolonic thiocolchicone and its derivatives, despite having the same substituents at C(7) [7].

In addition, the conformational equilibria of compounds with an *O*-aroyl side chain at C(7) (**15**, **15a,b**, **16**, **16a,b**, and **17**, **17a,b**) change more significantly on switching from $CHCl_3$ to DMSO or MeOH as solvent than those of compounds with an *O*-acyl side chain at C(7). Compounds with substituents at C(7) also show variable solvent-dependent optical rotations. For the alcohols (**11a,b**) and compounds with an *O*-acyl substituent at C(7) (**13a,b**, **14a,b**, and **19a,b**), the optical rotations change very little in solvents from $CHCl_3$ to MeOH. However, for compounds with an *O*-aroyl substituent at C(7) (**15a,b**, **16a,b**, and **17a,b**), the optical rotations change significantly in comparison with the alcohols [1]. (7*S*)-Compounds with a negative rotation in $CHCl_3$ shift to a positive value in MeOH, and (7*R*)-compounds with a positive rotation in $CHCl_3$ shift to a negative value in MeOH. These data suggest a conformational conversion of (a*S*) to (a*R*) in (7*S*)-*O*-aroyl compounds and of (a*R*) to (a*S*) in (7*R*)-*O*-aroyl compounds, in MeOH.

Biological Results. – The newly synthesized compounds were evaluated for inhibitory effects on tubulin polymerization, and contemporaneous comparative studies were also performed with thiocolchicine (**2**), allocolchicine (**3**), *N*-acetylcolchicinol *O*-methyl ether (**4**), and thiocolchicone (**6**). Selected compounds were also examined for their ability to inhibit the binding of [^{3}H]colchicine to tubulin when present at an equimolar concentration with the radiolabeled ligand. The data obtained are summarized in *Table 1*.

Almost all compounds had strong inhibitory effects on the polymerization reaction, with IC_{50} values over a relatively narrow range, from 1.7 to 5.1 μM. Weaker inhibition occurred with compound **11b**, which had an IC_{50} value of 9.0 μM. A few agents had negligible effects on polymerization, yielding IC_{50} values greater than 40 μM. These inactive compounds were the lactams **7** and **8**, the camphanates **12a** and **12b**, the cyclohexanecarboxylates **19**, **19a**, and **19b**, and, most notably, the (7*S*)-benzoate **15a**. For the most part, the agents causing strongest inhibition of polymerization also caused the greatest inhibition of [^{3}H]colchicine binding.

The allothiocolchicinoid ketone **9**, like the thiocolchicinoid ketone **6**, had activity comparable to that of thiocolchicine. It was among the ester isomers that unexpected results were obtained; in most cases significant activity was observed in *both* the (7*S*)- and (7*R*)-enantiomers.

In the previous study [7], we had observed apparent 8.3-fold greater inhibition of tubulin polymerization activity in the (7*S*)-alcohol as compared with the (7*R*)-alcohol, 4.3-fold greater activity in the (7*S*)-acetate, and 17-fold greater activity in the (7*S*)-camphanate (note that the allothiocolchicinoid camphanates are inactive). In the earlier study, we had prepared only the racemic and (7*S*)-nicotinates and (7*S*)-isonicotinates,

Table 1. *Interactions of Allocolchicinoids with Tubulin*

Compound	Tubulin polymerization[a]) $IC_{50} \pm$ SD (μM)	Colchicine-binding[b]) inhibition [%]
2a	2.1 ± 0.4	44
3	2.3 ± 0.05	70
4	2.1 ± 0.08	86
6	2.1 ± 0.09	74
7	> 40	–
8	> 40	–
9	1.7 ± 0.08	87
10	2.1 ± 0.06	89
11	3.3 ± 0.2	–
11a	1.8 ± 0.4	95
11b	9.0 ± 0.3	28
12a	> 40	–
12b	> 40	–
13a	2.1 ± 0.4	84
13b	3.1 ± 0.5	62
14	2.4 ± 0.4	–
14a	2.8 ± 0.2	76
14b	4.8 ± 0.06	51
15	3.3 ± 0.7	–
15a	> 40	18
15b	2.7 ± 0.4	44
16	2.5 ± 0.2	...
16a	4.8 ± 0.7	34
16b	2.4 ± 0.3	70
17	3.1 ± 0.2	–
17a	5.1 ± 0.5	41
17b	2.3 ± 0.2	74
18	3.5	–
19	> 40	...
19a	> 40	–
19b	> 40	–

[a]) Reaction mixtures contained 0.8M monosodium glutamate (pH 6.6 with HCl), 0.4 mM GTP, 1.0 mg/ml (10 μM) tubulin, 4% (*v*/*v*) DMSO, and various drug concentrations. All components except GTP were preincubated for 15 min at 30°. Reaction mixtures were chilled on ice, GTP was added, and the mixtures were transferred to cuvettes held at 0° in recording spectrophotometers. Polymerization was followed for 20 min at 30°, with turbidity measured at 350 nm. IC_{50} Values represent the concentration inhibiting extent of assembly to 50%. Values from three independent experiments were averaged.

[b]) Reaction mixtures contained 0.1 mg/ml (1.0 μM) tubulin, 5 μM [ring-A-4-^{3}H]colchicine, and the indicated inhibitor at 5 μM. Incubation was for 10 min at 37°. Values obtained from two independent experiments were averaged.

but their relative activities were also consistent with the (7*S*)-enantiomers having the greater activity (*Table 2*) as inhibitors of tubulin polymerization.

In the current allothiocolchicinoid study, in most cases there are only small differences between the two enantiomers of each pair. The major exceptions are the alcohols **11a** and **11b**, where the (7*S*)-isomer is 5.0-fold more active than the (7*R*)-isomer, and the benzoates **15a** and **15b**, where the (7*R*)-isomer appears to be at least 15-fold more inhibitory than the (7*S*)-isomer (*Table 1*). Even in the case of the alcohols, the activity

Table 2. *Relative Isomer Activities Compared in the Thiocolchicinoid and Allothiocolchicinoid-Ester Series* (ratio of IC_{50} values)

Acyl group at	Thiocolchicinoids[a])		Allothiocolchicinoids[b])	
C(7)	(7*R*)/(7*S*)	Racemate/(7*S*)	(7*R*)/(7*S*)	Racemate/(7*S*)
None (alcohol)	8.3	1.6	5.0	1.8
Acetate	4.3	2.2	1.5	n.a.[c])
Butyrate	n.a.	1.8	1.7	0.9
Camphanate	12	n.a.	n.m.[d])	n.a.
Nicotinate	n.a.	1.1	0.5	0.5
Isonicotinate	n.a.	2.0	0.5	0.5

[a]) Data from [7]. [b]) Data from *Table 1*. [c]) n.a.: Not available. [d]) n.m.: Not meaningful.

difference between the enantiomers is smaller with the allothiocolchicinoid pair than with the thiocolchicinoid pair (*Table 2*).

With the *O*-acyl pairs, the (7*S*)-acetate **13a** was 1.5 times as active as the (7*R*)-acetate **13b**, and the (7*S*)-butyrate **14a** 1.7 times as active as the (7*R*)-butyrate **14b**. With the *O*-aroyl pairs, the (7*R*)-nicotinate **16b** was 2.0 times as active as the (7*S*)-nicotinate **16a**, and the (7*R*)-isonicotinate **17b** 2.2 times as active as the (7*S*)-isonicotinate **17a**. With each enantiomer pair, the [^{3}H]colchicine-binding assay gave results in agreement with the polymerization assay.

In summary, in most cases in this series of allothiocolchicinoid ester derivatives both the (7*S*)- and (7*R*)-enantiomers had significant inhibitory interactions with tubulin. Among the available compounds, the (7*S*)-*O*-acyl derivatives are more active than their (7*R*)-enantiomers, while the (7*R*)-*O*-aroyl derivatives are more active than their (7*S*)-counterparts.

Discussion. – The X-ray crystallographic data confirmed the (a*R*,7*R*)-conformation for compound **12b** and, by extension, for the agents derived therefrom (**11b**, **13b**–**17b**, and **19b**) and the (a*S*,7*S*)-conformation for the enantiomeric agents **11a**, **13a**–**17a**, and **19a** derived from **12a**. However, in many cases significant interactions with tubulin occurred with both members of an enantiomeric pair. Moreover, the (7*R*)-isomers **15b**–**17b** were more potent inhibitors than their (7*S*)-enantiomers. We, therefore, performed a more extensive NMR analysis of these compounds in a variety of organic solvents, and we obtained convincing evidence for the *O*-aroyl compounds that increasing solvent polarity resulted in increasing proportions of the (a*S*,7*R*)- or (a*R*,7*S*)-conformer in solution at the expense of the opposite biaryl conformer. It thus seems likely that, in aqueous solution, significant amounts of both biaryl conformers exist with most if not all the allothiocolchicinoid derivatives, even the *O*-acyl esters and the alcohols. This would account for the loss of chiral specificity in this series of colchicine analogs, while retaining this explanation for the substantially greater activity of (–)-7*S*-colchicine as compared with (+)-(7*R*)-colchicine [4].

The more readily documented solvent-dependent conformer equilibration with the *O*-aroyl allothiocolchicinoids suggests that hydrophobic π-π stacking interactions may be an important factor in this phenomenon. For example, with a (7*S*)-*O*-aroyl derivative, an interaction between the side-chain aromatic residue and the aromatic *A* ring would

impose an (a*R*)-configuration on the molecule and at the same time stabilize this configuration. This result was further supported by molecular modeling study in Conformational Search and Molecular Dynamics Simulation [1]. Such an interaction should be enhanced in polar solvents, such as the aqueous media used in the biochemical assays. Thus, a π-π interaction could overcome the energetically favored equatorial orientation imposed on the side chain by the (a*S*)-biaryl configuration and permit the equilibrium to favor the (a*R*)-configuration with the less favored axially oriented side chain. The opposite would be the case with a (7*R*)-*O*-aroyl derivative, resulting in the apparent reversal of chiral preference we observed with compounds **15**–**17**. Since we found no evidence for such a chiral reversal in our previous study of 7-*O*-thiocolchicine derivatives [7], such an (a*S*) $\rightleftharpoons$ (a*R*) equilibration must occur more readily in biphenyls than in phenyltropolones.

In conclusion, the 7-*O*-allothiocolchicine derivatives, particularly those with *O*-aroyl groups, are exceptions to the generalization that colchicinoids, thiocolchicinoids, and allocolchicinoids with a (7*S*)- or (7*R*)-configuration energetically favor an (a*S*)- or (a*R*)-biaryl configuration, respectively, to maintain the side chain at C(7) in an equatorial orientation. Being able to readily assume both biaryl configurations, members of this family of compounds have little chiral specificity in their interaction with tubulin, and, in some cases, the (7*R*)-enantiomers are moderately more active than the (7*S*)-enantiomers.

Experimental Part

General. TLC: silica-gel plates, from *Analtech Inc.* CC: Silica gel (230–400 mesh) from *Aldrich.* M.p.: *Fisher-Johns* melting-point apparatus without correction. Optical rotations: *DIP-1000* polarimeter. UV Spectra: *UV 2101* spectrophotometer, in CH_2Cl_2. ^{1}H-NMR Spectra[1]): *Bruker AC-300* spectrometer, with Me_4Si (TMS) as the internal reference and $CDCl_3$ as solvent. Elemental analyses were performed by *Atlantic Microlab Inc.*, Norcross, GA. MS: determined by NIH.

Starting Material. Thiocolchicone (= *5,6,7,9-tetrahydro-1,2,3-trimethoxy-10-(methylthio)benzo[*a*]heptalene-7,9-dione*, **6**) was prepared according to the procedure reported in [7] in a 76.6% yield. M.p. 233–235°. $[\alpha]_D^{25} = 0$ ($c = 0.32$, MeOH). UV (CH_2Cl_2): λ_{max} 370 (4.25), 261 (4.35), 228 (4.25).

*5,5a,6,7-Tetrahydro-5a-hydroxy-9,10,11-trimethoxy-3-(methylthio)-4*H*-benzo[4,5]cyclohepta[1,2,3-*cd*]isoindol-4-one* (**7**). Compound **6** (50.7 mg, 0.136 mmol) was dissolved in 10% $MeNH_2$ soln. in benzene (6 ml). The mixture was refluxed for 3 h and monitored by TLC. The soln. was concentrated, and the residue was chromatographed on prep. TLC plates using Et_2O/hexane 8:1 to afford a red solid. Crystallization from acetone/MeOH and recrystallization from acetone gave 12 mg of **7** as colorless crystals. M.p. 179–182°. $[\alpha]_D^{25} = 0$. ^{1}H-NMR ($CDCl_3$)[1]): 1.85–2.80 (*m*, 2 H–C(5,6)); 2.57 (*s*, MeS); 3.04 (*s*,MeN); 3.65 (*s*, MeO–C(1)); 3.91 (*s*, MeO–C(3)); 3.93 (*s*, MeO–C(2)); 6.68 (*s*, H–C(4)); 7.27 (*d*, $J = 8.3$, H–C(11)); 7.72 (*d*, $J = 8.3$, H–C(12)). CI-MS: 402 (M^+). Anal. calc. for $C_{17}H_{23}NO_5S$ (353.43): C 57.77, H 6.56, N 3.96, S 9.07; found: C 57.61, H 6.78, N 4.03, S 9.15.

*5-Butyl-5,5a,6,7-tetrahydro-5a-hydroxy-9,10,11-trimethoxy-3-(methylthio)-4*H*-benzo[4,5]cyclohepta[1,2,3-*cd*]isoindol-4-one* (**8**). Preparation procedure was the same as for **7**, starting with 63.2 mg (0.17 mmol) of **6** and 3 mol of $BuNH_2$ in 4 ml of benzene. After TLC using AcOEt/hexane 1:2 and crystallization from Et_2O/MeOH, 15 mg of **8** was obtained. Colorless needles. M.p. 163–165°. ^{1}H-NMR ($CDCl_3$)[1]): 0.95 (*t*, $J = 7.5$, $MeCH_2$): 1.38 (*sext.*, $J = 7.5$, $MeCH_2$); 1.63 (*quint.*, $J = 7.5$, CH_2CH_2N); 2.60 (*s*, MeS); 3.44 (*s*, MeO–C(1)); 3.70 (*t*, $J = 7.5$, CH_2N); 3.92 (*s*, MeO–C(3)); 3.94 (*s*, MeO–C(2)); 6.66 (*s*, H–C(4)); 7.83 (*d*, $J = 8.5$, H–C(11)); 8.28 (*d*, $J = 8.5$, H–C(12)). CI-MS: 443 ([M – H]$^+$).

*6,7-Dihydro-9,10,11-trimethoxy-3-(methylthio)-5*H*-dibenzo[*a*,*c*]cycloheptene-5-one* (**9**). Compound **6** (1612.5 mg, 4.335 mmol) was dissolved in 50% $PhNH_2$ soln. in benzene (60 ml). The reaction was allowed to

[1]) NMR Assignments: according to the *trivial* C-atom numbering as in [6][7].

reflux and monitored by TLC. After the reaction was complete, the mixture was concentrated, and the residue was chromatographed on a flash column using hexane/AcOEt 9:1 as eluant. The combined product eluate was concentrated to give a colorless solid, which was crystallized from CH_2Cl_2/MeOH and recrystallized from CH_2Cl_2 to yield **9** (580 mg, 39.2%). Colorless prisms. M.p. 165–166°. $[\alpha]_D^{25} = 0$ ($c = 0.35$, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 286 (4.36), 251 (4.27), 231 (4.38). ^{1}H-NMR ($CDCl_3$)[1]): 2.55 (*s*, MeS); 2.60–3.10 (*m*, 2 H–C(5,6)); 3.52 (*s*, MeO–C(1)); 3.90 (*s*, MeO–C(3)); 3.91 (*s*, MeO–C(2)); 6.61 (*s*, H–C(4)); 7.38 (*d*, $J = 2.0$, H–C(8)); 7.40 (*dd*, $J = 8.9$, 2.0, H–C(10)); 7.51 (*d*, $J = 8.9$, H–C(11)). ^{13}C-NMR ($CDCl_3$)[1]): 15.4 (MeS); 30.0 (C(5)); 47.9 (C(6)); 56.0 (MeO–C(3)); 60.9 (MeO–C(1)); 61.1 (MeO–C(2)); 107.1 (C(4)); 123.9 (C(1a)); 124.6 (C(8)); 128.6 (C(11)); 130.6 (C(2)); 131.6 (C(10)); 135.7 (C(4a), C(11a)); 138.2 (C(9)); 139.8 (C(1)); 152.2 (C(7a)); 153.1 (C(3)); 206.5 (C(7)). CI-MS: 362 ([M + H + NH_3]$^+$). Anal. calc. for $C_{19}H_{20}O_4S$ (344.40): C 66.26, H 5.85, S 9.31; found: C 66.38, H 5.96, S 9.47.

*6,7-Dihydro-9,10,11-trimethoxy-3-(methylthio)-5*H*-dibenzo[*a,c*]cyclohepten-5-one Oxime* (**10**). To a soln. of **9** (13.2 mg, 0.038 mmol) in CH_2Cl_2 were added $NH_2OH \cdot HCl$ and AcONa in MeOH. This mixture was refluxed for 8–10 h and monitored by TLC. After concentration, the residue was purified by TLC with AcOEt/hexane 1:2: **10** (12 mg, 88.1%). White solid. M.p. 214–216°. ^{1}H-NMR[1]): 2.54 (*s*, MeS); 2.85–3.34 (*m*, 2 H–C(5,6)); 3.52 (*s*, MeO–C(1)); 3.90 (*s*, MeO–C(2,3)); 6.58 (*s*, H–C(4)); 7.31 (*s*, H–C(8)); 7.32 (*dd*, $J = 1.5$, 8.0, H–C(10)); 7.48 (*d*, $J = 8.0$, H–C(11)). ^{13}C-NMR ($CDCl_3$)[1]): 15.5 (MeS); 30.0 (C(5)); 47.9 (C(6)); 56.0 (MeO–C(3)); 60.9 (MeO–C(1)); 61.1 (MeO–C(2)); 107.2 (C(4)); 124.2 (C(1a)); 126.0 (C(8)); 126.6 (C(1)); 131.5 (C(10)); 131.8 (C(7a)); 135.2 (C(2)); 136.2 (C(4a)); 137.5 (C(11a)); 141.3 (C(9)); 151.8 (C(1)); 152.8 (C(3)); 161.4 (C(7)). CI-MS: 377 ([M + H + NH_3]$^+$). Anal. calc. for $C_{19}H_{21}NO_4S$ (359.44): C 63.49, H 5.89, N 3.90, S 8.92; found: C 63.54, H 6.01, N 3.98, S 9.12.

*(±)-6,7-Dihydro-9,10,11-trimethoxy-3-(methylthio)-5*H*-dibenzo[*a,c*]cyclohepten-5-ol* (**11**). To a soln. of **9** (680.4 mg, 1.98 mmol) in CH_2Cl_2/MeOH was added $NaBH_4$ (320 mg, 8.46 mmol) at −78°, and the soln. was stirred at −78° to 0° overnight. The mixture was acidified with 50% AcOH, then extracted with CH_2Cl_2 (4 × 10 ml). The combined org. phases were washed with sat. NaCl, dried (Na_2SO_4), and concentrated to give a residue (623 mg), which was crystallized from Et_2O/CH_2Cl_2 and recrystallized twice from Et_2O/CH_2Cl_2 to yield pure **11** (709.4 mg, 90.8%). Colorless prisms. M.p. 177°. $[\alpha]_D^{24.5} = 0$ ($c = 0.31$, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 283 (4.48), 230 (4.38). ^{1}H-NMR ($CDCl_3$)[1]): major conformer: 1.87–2.62 (*m*, 2 H–C(5,6)); 2.57 (*s*, MeS); 3.62 (*s*, MeO–C(1)); 3.92 (*s*, MeO–C(2)); 3.91 (*s*, MeO–C(3)); 4.62 (*dd*, $J = 7.0$, 10.8, H–C(7)); 6.60 (*s*, H–C(4)); 7.23 (*dd*, $J = 2.1$, 8.0, H–C(10)); 7.40 (*d*, $J = 8.0$, H–C(11)); 7.60 (*d*, $J = 2.1$, H–C(8)); minor conformer: 1.87–2.62 (*m*, 2 H–C(5,6)); 2.55 (*s*, MeS); 3.61 (*s*, MeO–C(1)); 3.91 (*s*, MeO–C(3)); 3.92 (*s*, MeO–C(2)); 4.72 (*d*, $J = 5.0$, H–C(7)); 6.66 (*s*, H–C(4)); 7.23 (*dd*, $J = 2.1$, 8.0, H–C(10)); 7.40 (*d*, $J = 8.0$, H–C(11)); 7.60 (*d*, $J = 2.1$, H–C(8)). CI-MS: 364 ([M + H + NH_3]$^+$). Anal. calc. for $C_{19}H_{22}O_4S$ (346.44): C 65.87, H 6.40, S 9.25; found: C 65.91, H 6.33, S 9.37.

General Procedure for Synthesizing Racemic Esters **12–19** *and Enantiomers* **13a,b–19a,b**. To a soln. of the corresponding alcohol, **11**, **11a**, or **11b**, in dry pyridine was added an appropriate acyl or aroyl chloride or anhydride (1.5–2.0 equiv.) at 0° or r.t. The mixture was stirred and allowed to stand overnight, and the volatile solvents were removed *in vacuo*. The residue was diluted with CH_2Cl_2 or Et_2O, then filtered, and the filtrate was washed with CH_2Cl_2 or Et_2O several times. After concentration *in vacuo*, the residue was submitted to prep. TLC or FC, or purified by crystallization.

*(±)-6,7-Dihydro-9,10,11-trimethoxy-3-(methylthio)-5*H*-dibenzo[*a,c*]cyclohepten-5-yl Butanoate* (**14**). Yield 30.4%. M.p. 103–105°. $[\alpha]_D^{25} = 0$ ($c = 0.36$, $CHCl_3$). ^{1}H-NMR[1]): major conformer: 0.99 (*t*, MeCH_2); 1.71 (*sext.*, MeCH_2); 2.47 (*t*, CH_2CO); 2.55 (*s*, MeS); 1.99–2.62 (*m*, 2 H–C(5,6)); 3.58 (*s*, MeO–C(1)); 3.91 (*s*, MeO–C(3)); 3.93 (*s*, MeO–C(2)); 5.60 (*dd*, $J = 7.0$, 10.8, H–C(7)); 6.59 (*s*, MeO–C(4)); 7.23 (*dd*, $J = 1.8$, 8.0, H–C(10)); 7.43 (*d*, $J = 8.0$, H–C(11)); 7.35 (*d*, $J = 1.8$, H–C(8)); minor conformer: 0.72 (*t*, *Me*CH_2); 1.85 (*sext.*, MeCH_2); 2.47 (*t*, CH_2CO); 2.54 (*s*, MeS); 1.99–2.62 (*m*, 2 H–C(5,6)); 3.62 (*s*, MeO–C(1)); 3.91 (*s*, MeO–C(3)); 3.93 (*s*, MeO–C(2)); 5.80 (*d*, $J = 5.2$, H–C(7)); 6.61 (*s*, H–C(4)); 7.20 (*dd*, $J = 1.8$, 8.0, H–C(10)); 7.49 (*d*, $J = 8.0$, H–C(11)); 7.28 (*d*, $J = 1.8$, H–C(8)). CI-MS: 434 ([M + H + NH_3]$^+$). Anal. calc. for $C_{23}H_{28}O_5S$ (416.53): C 66.32, H 6.75, S 7.70; found: C 66.19, H 6.85, S 7.55.

*(±)-6,7-Dihydro-9,10,11-trimethoxy-3-(methylthio)-5*H*-dibenzo[*a,c*]cyclohepten-5-yl Benzoate* (**15**). Yield 97% (starting with 50.5 mg of **11**). White solid. M.p. 125–128°. $[\alpha]_D^{29} = 0$ ($c = 0.26$, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 281 (4.48), 231 (4.57). ^{1}H-NMR ($CDCl_3$)[1]): major conformer: 2.51 (*s*, MeS); 2.15–2.75 (*m*, 2 H–C(5,6)); 3.61 (*s*, MeO–C(1)); 3.93 (*s*, MeO–C(3)); 3.95 (*s*, MeO–C(2)); 5.84 (*dd*, $J = 7.2$, 10.8, H–C(7)); 6.63 (*s*, H–C(4)); 7.48 (*d*, $J = 8.0$, H–C(10)); 7.51 (*d*, $J = 8.0$, H–C(11)); 7.46 (*s*, H–C(8)); 7.28 (*m*, H–C(3,5)); 7.62 (*t*, $J = 7.0$, H–C(4) of Ph); 8.15 (*d*, $J = 7.0$, H–C(2,6) of Ph); minor conformer: 2.56 (*s*, MeS); 2.15–2.75 (*m*, 2 H–C(5,6)); 3.23 (*s*, MeO–C(1)); 3.90 (*s*, MeO–C(3)); 3.95 (*s*, MeO–C(2)); 6.07 (*d*, $J = 5.1$, H–C(7));

6.71 (*s*, H–C(4)); 7.48 (*d*, *J* = 8.0, H–C(10)); 7.53 (*d*, *J* = 8.0, H–C(11)); 7.45 (*s*, H–C(8)); 7.28 (*m*, H–C(3,5) of Ph); 7.41 (*t*, *J* = 7.0, H–C(4') of Ph); 7.52 (*d*, *J* = 7.0, H–C(2,6) of Ph). CI-MS: 468 ($[M + H + NH_3]^+$). Anal. calc. for $C_{26}H_{26}O_5S$ (450.54): C 69.63, H 5.82, S 7.12; found: C 69.74, H 5.68, S 7.08.

*(±)-6,7-Dihydro-9,10,11-trimethoxy-3-(methylthio)-5*H*-dibenzo[a,c]cyclohepten-5-yl Pyridine-3-carboxylate* (**16**). Yield 53.2% (starting with 26.7 mg of **11**). M.p. 147–148°. $[\alpha]_D^{25} = 0$ (*c* = 0.25, $CHCl_3$). ^{1}H-NMR ($CDCl_3$)[1]): major conformer: 2.51 (*s*, MeS); 2.14–2.75 (*m*, 2 H–C(5,6)); 3.61 (*s*, MeO–C(1)); 3.93 (*s*, MeO–C(3)); 3.94 (*s*, MeO–C(2)); 5.86 (*dd*, *J* = 7.2, 10.8, MeO–C(7)); 6.63 (*s*, H–C(4)); 7.28 (*dd*, *J* = 1.5, 8.0, H–C(10)); 7.47 (*d*, *J* = 8.0, H–C(11)); 7.41 (*s*, H–C(8)); 7.46 (*s*, H–C(5) of Py); 8.85 (*s*, H–C(4) of Py); 8.38 (*d*, *J* = 8.0, H–C(6) of Py); 9.37 (*s*, H–C(2) of Py); minor conformer: 2.56 (*s*, MeS); 2.14–2.75 (*m*, H–C(5,6)); 3.62 (*s*, MeO–C(1)); 3.91 (*s*, MeO–C(3)); 3.94 (*s*, MeO–C(2)); 6.08 (*d*, *J* = 5.0, H–C(7)); 6.69 (*s*, H–C(4)); 7.28 (*dd*, *J* = 1.5, 8.0, H–C(10)); 7.52 (*d*, *J* = 8.0, H–C(11)); 7.29 (*s*, H–C(8)); 7.26 (*s*, H–C(5) of Py); 7.20 (*s*, H–C(4) of Py); 7.61 (*d*, *J* = 8.0, H–C(6) of Py); 8.64 (*s*, H–C(2) of Py). CI-MS: 452 ($[M + H]^+$). Anal. calc. for $C_{25}H_{25}NO_5S$ (451.53): C 66.50, H 5.58, N 3.10, S 7.10; found: C 66.67, H 5.45, N 3.20, S 7.18.

*(±)-6,7-Dihydro-9,10,11-trimethoxy-3-(methylthio)-5*H*-dibenzo[a,c]cyclohepten-5-yl Pyridine-3-carboxylate* (**17**). Yield 72.9% (starting with 69.7 mg of **11**). Crystallization from Et_2O/acetone afforded colorless needles. M.p. 167–167.5°. $[\alpha]_D^{25} = 0$ (*c* = 0.31, $CHCl_3$). ^{1}H-NMR ($CDCl_3$)[1]): major conformer: 2.51 (*s*, MeS); 2.13–2.77 (*m*, 2 H–C(5,6)); 3.62 (*s*, MeO–C(1)); 3.93 (*s*, MeO–C(3)); 3.95 (*s*, MeO–C(2)); 5.85 (*dd*, *J* = 7.2, 10.8, H–C(7)); 6.63 (*s*, H–C(4)); 7.26 (*d*, *J* = 8.0, H–C(10)); 7.48 (*d*, *J* = 8.0, H–C(11)); 7.38 (*s*, H–C(8)); 7.96 (*s*, H–C(3,5) of Py); 8.86 (*s*, H–C(2,6) of Py); minor conformer: 2.56 (*s*, MeS); 2.13–2.77 (*m*, H–C(5,6)); 3.31 (*s*, MeO–C(1)); 3.90 (*s*, MeO–C(3)); 3.95 (*s*, MeO–C(2)); 6.08 (*d*, *J* = 5.0, H–C(7)); 6.70 (*s*, H–C(4)); 7.28 (*d*, *J* = 8.0, H–C(10)); 7.52 (*d*, *J* = 8.0, H–C(11)); 7.28 (*s*, H–C(8)); 7.21 (*s*, H–C(3,5) of Py); 8.61 (*s*, H–C(2,6) of py). CI-MS: 452 ($[M + H]^+$). Anal. calc. for $C_{25}H_{25}NO_5S$ (451.53): C 66.50, H 5.58, N 3.10, S 7.10; found: C 66.45, H 5.62, N 3.18, S 7.06.

*(±)-6,7-Dihydro-9,10,11-trimethoxy-3-(methylthio)-5*H*-dibenzo[a,c]cyclohepten-5-yl Trifluoroacetate* (**18**). Yield 95.2% (starting with 28.1 mg of **11**). Crystallization from Et_2O/hexane gave colorless needles. M.p. 134–135°. $[\alpha]_D^{25} = 0$ (*c* = 0.44, $CHCl_3$). ^{1}H-NMR ($CDCl_3$)[1]): major conformer: 2.13–2.71 (*m*, 2 H–C(5,6)); 2.54 (*s*, MeS); 3.60 (*s*, MeO–C(1)); 3.93 (*s*, MeO–C(3)); 3.94 (*s*, MeO–C(2)); 5.74 (*dd*, *J* = 7.2, 11.0, H–C(7)); 6.61 (*s*, H–C(4)); 7.28 (*d*, *J* = 8.5, H–C(10)); 7.46 (*d*, *J* = 8.5, H–C(11)); 7.29 (*s*, H–C(8)); minor conformer: 2.13–2.71 (*m*, 2 H–C(5,6)); 2.56 (*s*, MeS); 3.56 (*s*, MeO–C(1)); 3.89 (*s*, MeO–C(3)); 3.91 (*s*, MeO–C(2)); 5.93 (*d*, *J* = 5.5, H–C(7)); 6.58 (*s*, H–C(4)); 7.20 (*d*, *J* = 2.0, H–C(8)); 7.33 (*dd*, *J* = 2.0, 8.2, H–C(10)); 7.54 (*d*, *J* = 8.2, H–C(11)). CI-MS: 460 ($[M + H + NH_3]^+$). Anal. calc. for $C_{21}H_{21}O_5F_3S$ (442.44): C 57.01, H 4.78, S 7.25; found: C 57.12, H 4.86, S 7.14.

*(±)-6,7-Dihydro-9,10,11-trimethoxy-3-(methylthio)-5*H*-dibenzo[a,c]cyclohepten-5-yl Cyclohexanecarboxylate* (**19**). Yield 60.7% (starting with 35.4 mg of **11**). M.p. 117–118°. $[\alpha]_D^{25} = 0$ (*c* = 0.30, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 282.2 (4.31), 229.6 (4.19). ^{1}H-NMR ($CDCl_3$)[1]): major conformer: 2.55 (*s*, MeS); 1.94–2.61 (*m*, 2 H–C(5,6)); 3.58 (*s*, MeO–C(1)); 3.91 (*s*, MeO–C(3)); 3.92 (*s*, MeO–C(2)); 5.56 (*dd*, *J* = 6.80, 11.0, H–C(7)); 6.58 (*s*, H–C(4)); 7.22 (*dd*, *J* = 1.8, 8.1, H–C(10)); 7.43 (*d*, *J* = 8.1, H–C(11)); 7.33 (*d*, *J* = 1.8, H–C(8)); 1.18–2.04 (*m*, 9 H, cyclohexyl); minor conformer: 2.54 (*s*, MeS); 1.94–2.61 (*m*, 2 H–C(5,6)); 3.62 (*s*, MeO–C(1)); 3.90 (*s*, MeO–C(3)); 3.92 (*s*, MeO–C(2)); 5.80 (*d*, *J* = 5.5, H–C(7)); 6.58 (*s*, H–C(4)); 7.19 (*d*, *J* = 8.0, H–C(10)); 7.50 (*d*, *J* = 8.0, H–C(11)); 7.29 (*s*, H–C(8)); 1.18–2.04 (*m*, 9 H, cyclohexyl). Anal. calc. for $C_{26}H_{32}O_5S$ (456.59): C 68.39, H 7.06, S 7.02; found: C 68.44, H 7.12, S 7.08.

(–)-Camphanate (**12a**). Yield 24.5%. Resolved from diastereoisomer mixture *(±)-6,7-Dihydro-9,10,11-trimethoxy-3-(methylthio)-5*H*-dibenzo[a,c]cyclohepten-5-yl 4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylate* (**12**) first by FC $CH_2Cl_2/CHCl_3$ 1:1, then by prep. TLC with hexane/AcOEt 2:1. Colorless oil. $[\alpha]_D^{25} = -100$ (*c* = 0.30, $CHCl_3$). ^{1}H-NMR ($CDCl_3$)[1]): 0.99 (*s*, Me of camphanoyl); 1.08 (*s*, Me of camphanoyl); 1.15 (*s*, Me of camphanoyl); 1.90–2.12 (*m*, 2 H–C(4,5) of camphanoyl); 2.27–2.61 (*m*, 2 H–C(5,6)); 2.47 (*s*, MeS); 3.68 (*s*, MeO–C(1)); 3.93 (*s*, MeO–C(3)); 3.95 (*s*, MeO–C(2)); 5.43 (*dd*, *J* = 6.0, 10.9, H–C(7)); 6.58 (*s*, H–C(4)); 7.08 (*d*, *J* = 10.5, H–C(10)); 7.31 (*d*, *J* = 10.5, H–C(11)); 8.31 (*s*, H–C(8)). CI-MS: 544 ($[M + H + NH_3]^+$). Anal. calc. for $C_{29}H_{34}O_7S$ (526.64): C 66.14, H 6.51, S 6.09; found: C 66.11, H 6.61, S 6.10.

(+)-Camphanate (**12b**). Yield 21.3%. Resolved from diastereoisomer mixture **12** first by FC after the **12a** fraction, using $CHCl_3/CH_2Cl_2$ 1:1, then by prep. TLC with hexane/AcOEt 2:1. Crystallization from CH_2Cl_2 gave colorless crystals. M.p. 166–168°. $[\alpha]_D^{25} = +131$ (*c* = 0.34, $CHCl_3$). ^{1}H-NMR ($CDCl_3$)[1]): 1.02, 1.10, 1.15 (3*s*, 3 Me of camphanoyl); 1.19–2.17 (*m*, 2 H–C(4,5) of camphanoyl); 2.36–2.63 (*m*, 2 H–C(5,6)); 2.47 (*s*, MeS); 3.67 (*s*, MeO–C(1)); 3.93 (*s*, MeO–C(3)); 3.95 (*s*, MeO–C(2)); 5.48 (*dd*, *J* = 6.5, 10.8, H–C(7)); 6.58 (*s*, H–C(4)); 7.11 (*d*, *J* = 10.5, H–C(10)); 7.33 (*d*, *J* = 10.5, H–C(11)); 7.32 (*s*, H–C(8)). CI-MS: 544 ($[M + H + NH_3]^+$). Anal. calc. for $C_{29}H_{34}O_7S$ (526.64): C 66.14, H 6.51, S 6.09; found: C 66.11, H 6.61, S 6.10.

*Compound (−)-***11a**. To a soln. of **12a** (158 mg, 0.3 mmol) in a mixture of MeOH/CH_2Cl_2 1:1 (5 ml) was added 2N NaOH (1.5 ml) at −78°. The soln. was stirred at −78° for 0.5 h, then at r.t. for 2 h, and monitored by TLC. The mixture was extracted with CH_2Cl_2, (3 × 10 ml), and the combined CH_2Cl_2 layer was washed with H_2O (3 × 10 ml). The org. phase was dried (Na_2SO_4) and concentrated to give crude **11a**. Crystallization of **11a** afforded pure white solid: 105 mg (100%). M.p. 131–133°. $[\alpha]_D^{25} = -143.6$ ($c = 0.27$, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 282 (4.36), 229 (4.25). ^{1}H-NMR: identical with those of **11**. CI-MS: 364 ($[M + H + NH_3]^+$). Anal. calc. for $C_{19}H_{22}O_4S$ (346.44): C 65.87, H 6.4, S 9.25; found: C 65.81, H 6.43, S 9.30.

*Compound (+)-***11b**. Prepared as described for **11a**, starting with 125 mg (0.238 mmol) of **12b**. Crystallization from Et_2O/CH_2Cl_2 afforded a white powder: 76 mg (92.45%). M.p. 129.5–131°. $[\alpha]_D^{25} = +150.9$ ($c = 0.25$, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 282 (4.41), 2.29 (4.31). ^{1}H-NMR: identical with those of **11**. CI-MS: 364 ($[M + H + NH_3]^+$). Anal. calc. for $C_{19}H_{22}O_4S$ (346.44): C 65.87, H 6.40, S 9.25; found: C 65.85, H 6.53, S 9.32.

*Compound (−)-***13a**. Yield 79% (starting with 25.8 mg of **11a**). M.p. 107–108°. $[\alpha]_D^{25} = -146.9$ ($c = 0.51$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$)[1]: major conformer: 1.94–2.64 (*m*, 2 H–C(5,6)); 2.17 (*s*, MeCO); 2.55 (*s*, MeS); 3.58 (*s*, MeO–C(1)); 3.91 (*s*, MeO–C(3)); 3.93 (*s*, MeO–C(2)); 5.58 (*dd*, $J = 7.0$, 10.8, H–C(7)); 6.59 (*s*, H–C(4)); 7.23 (*dd*, $J = 1.8$, 8.0, H–C(10)); 7.43 (*d*, $J = 8.0$, H–C(11)); 7.35 (*d*, $J = 1.8$, H–C(8)); minor conformer: 1.94–2.64 (*m*, 2 H–C(5,6)); 2.17 (*s*, MeCO); 2.54 (*s*, MeS); 3.62 (*s*, MeO–C(1)); 3.91 (*s*, MeO–C(3)); 3.93 (*s*, MeO–C(2)); 5.77 (*d*, $J = 5.0$, H–C(7)); 6.59 (*s*, H–C(4)); 7.20 (*dd*, $J = 1.8$, 8.0, H–C(10)); 7.43 (*d*, $J = 8.0$, H–C(11)); 7.35 (*d*, $J = 1.8$, H–C(8)). CI-MS: 406 ($[M + H + NH_3]^+$). Anal. calc. for $C_{21}H_{24}O_5S$ (388.47): C 64.91, H 6.23, S 8.25; found: C 64.95, H 6.18, S 8.20.

*Compound (+)-***13b**. Yield 76.4% (starting with 16.2 mg of **11b**). M.p. 108–109°. $[\alpha]_D^{25} = +135.78$ ($c = 0.41$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): identical with those of **13a**. CI-MS: 406 ($[M + H + NH_3]^+$). Anal. calc. for $C_{21}H_{24}O_5S$ (388.47): C 64.91, H 6.23, S 8.25; found: C 64.98, H 6.20, S 8.32.

*Compound (−)-***14a**. Yield 36% (starting with 12.2 mg of **11a**). M.p. 114–115°. $[\alpha]_D^{25} = -114$ ($c = 0.53$, $CHCl_3$). ^{1}H-NMR: identical with those of **14**. Anal. calc. for $C_{23}H_{28}O_5S$ (416.53): C 66.32, H 6.75, S 7.70; found: C 66.20, H 6.82, S 7.67.

*Compound (+)-***14b**. Yield 52% (starting with 12.2 mg of **11b**). M.p. 114–115°. $[\alpha]_D^{25} = +110$ ($c = 0.53$, $CHCl_3$). ^{1}H-NMR: identical with those of **14**. Anal. calc. for $C_{23}H_{28}O_5S$ (416.53): C 66.32, H 6.75, S 7.70; found: C 66.35, H 6.70, S 7.76.

*Compound (−)-***15a**. Yield 94.5% (starting with 12.6 mg of **11a**). M.p. 134–136°. $[\alpha]_D^{29} = -12.6$ ($c = 0.30$, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 282 (4.34), 230.5 (4.51). ^{1}H-NMR: identical with those of **15**. Anal. calc. for $C_{26}H_{26}O_5S$ (450.54): C 69.63, H 5.82, S 7.12; found: C 69.62, H 5.85, S 7.10.

*Compound (+)-***15b**. Yield 86.8% (starting with 12.2 mg of **11b**). M.p. 136–137°. $[\alpha]_D^{29} = +13.2$ ($c = 0.32$, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 282 (4.51), 231 (4.54). ^{1}H-NMR: identical with those of **15**. Anal. calc. for $C_{26}H_{26}O_5S$ (450.54): C 69.63, H 5.82, S 7.12; found: C 69.78, H 5.75, S 7.16.

*Compound (−)-***16a**. Yield 90.3% (starting with 10.3 mg of **11a**). M.p. 120–122°. $[\alpha]_D^{25} = -35$ ($c = 0.59$, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 282 (4.37), 229 (4.40). ^{1}H-NMR: identical with those of **16**. Anal. calc. for $C_{25}H_{25}NO_5S$ (451.53): C 66.50, H 5.58, N 3.10, S 7.10; found: C 66.66, H 5.56, N 3.08, S 7.12.

*Compound (+)-***16b**. Yield 99.3% (starting with 11.1 mg of **11b**). M.p. 122–124°. $[\alpha]_D^{25} = +22.7$ ($c = 0.25$, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 282 (4.30), 229 (4.31). ^{1}H-NMR: identical with those of **16**. Anal. calc. for $C_{25}H_{25}NO_5S$ (451.53): C 66.50, H 5.58, N 3.10, S 7.10; found: C 66.58, H 5.60, N 3.18, S 7.04.

*Compound (−)-***17a**. Yield 81.5% (starting with 9.1 mg of **11a**). M.p. 133–134°. $[\alpha]_D^{25} = -15$ ($c = 0.485$, $CHCl_3$). ^{1}H-NMR: identical with those of **17**. Anal. calc. for $C_{25}H_{25}NO_5S$ (451.53): C 66.50, H 5.58, N 3.10, S 7.10; found: C 66.62, H 5.60, N 3.12, S 7.18.

*Compound (+)-***17b**. Yield 72.4% (starting with 7.5 mg of **11b**). M.p. 128–130°. $[\alpha]_D^{25} = +18.6$ ($c = 0.355$, $CHCl_3$). ^{1}H-NMR: identical with those of **17**. Anal. calc. for $C_{25}H_{25}NO_5S$ (451.53): C 66.50, H 5.58, N 3.10, S 7.10; found: C 66.48, H 5.60, N 3.16, S 7.05.

*Compound (−)-***19a**. Yield 95% (starting with 13.2 mg of **11a**). Oil. $[\alpha]_D^{25} = -123.6$ ($c = 0.30$, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 282.2 (4.25), 229.2 (4.14). ^{1}H-NMR: identical with those of **19**. Anal. calc. for $C_{26}H_{32}O_5S$ (456.59): C 68.39, H 7.06, S 7.02; found: C 68.34, H 7.10, S 7.08.

*Compound (+)-***19b**. Yield 93.8% (starting with 8.9 mg of **11b**). Oil. $[\alpha]_D^{25} = +116.1$ ($c = 0.30$, $CHCl_3$). UV (CH_2Cl_2): λ_{max} 282.2 (4.28), 229.6 (4.19). ^{1}H-NMR: identical with those of **19**. Anal. calc. for $C_{26}H_{32}O_5S$ (456.59): C 68.39, H 7.06, S 7.02; found: C 68.30, H 7.04, S 7.10.

X-Ray Crystal-Structure Analyses of Compounds **7** *and* **12b**. Crystal data for **7**: $C_{21}H_{23}NO_5S$, $M = 401.49$, monoclinic, space group $C2/c$ (C_{2h}^6), $a = 23.326(2)$ Å, $b = 9.652(1)$ Å, $c = 21.666(2)$ Å, $\beta = 125.98(1)°$, $V = 3947(2)$ Å^3, $Z = 8$, $D_{calc.} = 1.351$ g cm^{-3}, μ(CuK_α radiation) = 16.9 cm^{-1}; crystal dimensions: 0.20 × 0.30 × 0.30 mm. Crystal data for **12b**: $C_{29}H_{34}O_7S$, $M = 525.65$, monoclinic, space group $C2$(C_2^3),

$a = 31.845(5)$ Å, $b = 8.793(2)$ Å, $c = 9.952(2)$ Å, $\beta = 93.54(19)°$, $V = 2781(2)$ Å^3, $Z = 4$, $D_{calc.} = 1.258$ g cm^{-3}, μ(CuK_α radiation) = 13.6 cm^{-1}; crystal dimensions: $0.06 \times 0.10 \times 0.60$ mm.

Oscillation and *Weissenberg* photographs yielded preliminary unit-cell parameters and space group information. Intensity data ($+h$, $+k$, $\pm l$, 4173 reflections for **7**; $\pm h$, $-k$, $+l$, 3062 nonequivalent reflections for **12b**) were recorded on an *Enraf-Nonius CAD-4* diffractometer (CuK_α radiation, graphite monochromator; ω-2θ scans, scanwidths $(0.80 + 0.14 \tan\theta)°$ for **7**, and $(0.90 + 0.14 \tan\theta)°$ for **12b**); the intensities of four reference reflections, remeasured every 2 h, showed no significant variation (< 1%) throughout. Refined unit-cell parameters were calculated in each case from the diffractometer setting angles for 25 reflections ($36° < \theta < 40°$) widely separated in reciprocal space. Intensity data were corrected for the usual *Lorentz* and polarization effects; empirical absorption corrections, based on the ϕ-dependency of the intensities of several reflections with χ *ca.* 90°, were also applied (T_{max}/T_{min}(relative) = 1.00:0.89 for **7**, 1.00:0.91 for **12b**). Equivalent reflections were averaged for **7** (R_{merge}(on I) = 0.027) to yield 4058 independent values. Those 3207 and 2442 reflections with $I > 3.0\sigma(I)$ for **7** and **12b**, respectively, were retained for the structure analyses and parameter refinements. *Laue* symmetry indicated that crystals of **7** were monoclinic, space group *Cc* or *C*2/*c* (from the systematic absences: *hkl* when $h + k \neq 2n$; *h*0*l* when $l \neq 2n$); the centrosymmetric choice, *C*2/*c*, was assumed at the outset and shown to be correct by the structure solution and refinement. For **12b**, *Laue* symmetry revealed that the crystals were monoclinic; the space group *C*2 was established from the systematic absence (*hkl* when $h + k \neq 2n$) and the fact that **12b** is chiral.

Both crystal structures were solved by direct methods (MULTAN11/82) [13]. Initial coordinates for all non-H-atoms in **7** and **12b** were derived from an *E* map. For **12b**, the enantiomer was selected to yield the known absolute configuration of the camphanoyl moiety. Atomic positional and thermal parameters (first isotropic and then anisotropic) were adjusted by means of full-matrix least-squares calculations during which $\sum w\Delta^2$ [$w = 1/\sigma^2(|F_o|)$, $\Delta = (|F_o| - |F_c|)$] was minimized. H-Atoms were located in difference *Fourier* syntheses, and their positional and isotropic thermal parameters were refined in addition to the non-H-atom parameters in the subsequent least-squares iterations; an extinction correction (g) was included as a variable during the later cycles. The parameter refinements converged at $R\ (= \sum(\|F_o| - |F_c\|)/\sum|F_o|) = 0.037$, $\{R_w = [\sum w(|F_o| - |F_c|)^2/\sum w(|F_o|^2]^{1/2}) = 0.051$, $GOF = [\sum w\Delta^2/(N_{observations} - N_{parameters})]^{1/2} = 1.80$, $g = 1.3(1) \times 10^{-6}\}$ for **7**; $R = 0.041$ ($R_w = 0.056$), $GOF = 1.33$, $g = 1.2(3) \times 10^{-6}$ for **12b**. Final difference *Fourier* syntheses contained no unusual features ($\Delta\rho$(eÅ^{-3}) max., min.: 0.20–0.28 (**7**); 0.39–0.16 (**12b**)). Crystallographic calculations were performed on *PDP11/44* and *MicroVAX* computers by use of the *Enraf-Nonius* Structure Determination Package (SDP 3.0) [14]. For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from [15]; structure diagrams were prepared using the ORTEP-II program [16].

Crystallographic data (excluding structure factors) for the structure(s) reported in this paper have been deposited with the *Cambridge Crystallographic Data Centre* as supplementary publication. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 of 1EZ, UK. (fax: + 44-(0)1223-336033 or e-mail: teched@chemcrys.cam.ac.uk

Biological Assays. The tubulin polymerization and [^{3}H]colchicine binding assays were performed as described in [7].

This work was supported by grants *CA 17625* from the *National Cancer Institute* (*K. H. Lee*). We would like to thank Dr. *Xue-Feng Pei* (*NIH*) for MS measurements.

REFERENCES

[1] Q. Shi, K. Chen, X. Chen, A. Brossi, P. Verdier-Pinard, E. Hamel, A. T. McPhail, K. H. Lee, *J. Org. Chem.*, in press.
[2] M. Levy, M. Spino, S. E. Read, *Pharmacotherapy* **1991**, *11*, 196.
[3] G. J. Kang, Z. Getahun, A. Muzaffar, A. Brossi, E. Hamel, *J. Biol. Chem.* **1990**, *265*, 10255.
[4] H. J. C. Yeh, M. Chrzanowska, A. Brossi, *FEBS Lett.* **1988**, *229*, 82.
[5] A. Brossi, *Actual. Chim. Ther.* **1989**, *16*, 13.
[6] Q. Shi, P. Verdier-Pinard, A. Brossi, E. Hamel, K. H. Lee, *Bioorg. Med. Chem.* **1997**, *5*, 2277.
[7] Q. Shi, P. Verdier-Pinard, A. Brossi, E. Hamel, A. T. McPhail, K. H. Lee, *J. Med. Chem.* **1997**, *40*, 961.
[8] O. Boyé, A. Brossi, in 'The Alkaloids', Ed. A. Brossi, Academic Press, New York, 1983, Vol. 41, p. 125.
[9] O. Boyé, A. Brossi, H. J. C. Yeh, E. Hamel, B. Wegrzynski, V. Toome, *Can. J. Chem.* **1992**, *70*, 1237.
[10] Q. Shi, Ph. D. Thesis, University of North Carolina, 1997.
[11] M. G. Banwell, M. A. Fam, R. W. Gable, E. Hamel, *J. Chem. Soc., Chem. Commun.* **1994**, 2647.

[12] G. Muller, B. P. Vaterlaus, L. Velluz, *Bull. Soc. Chim. Fr.* **1957**, 434.
[13] MULTAN11'82, P. Main, S. J. Fiske, S. E. Hull, L. Lessinger, G. Germain, J. P. DeClerq, and M. M. Woolfson, Universities of York, England, and Louvain, Belgium, 1982.
[14] B. A. Frenz & Associates, Inc., SPD 3.0: Structure Determination Package, College Station, Texas, USA, and Enraf-Nonius, Delft, Holland, 1985.
[15] International Tables for X-Ray Crystallography, Eds. J. A. Ibers and W. C. Hamilton, The Kynoch Press, Birmingham, UK, 1974, Vol. IV.
[16] C. K. Johnson, 'ORTEP-II: Report ORNL-5138', Oak Ridge National Laboratory, Tennessee, USA, 1976.

Received December 22, 1997