Covalent Reversible Binding of Alkoxides or Thiolates to Colchicinoids

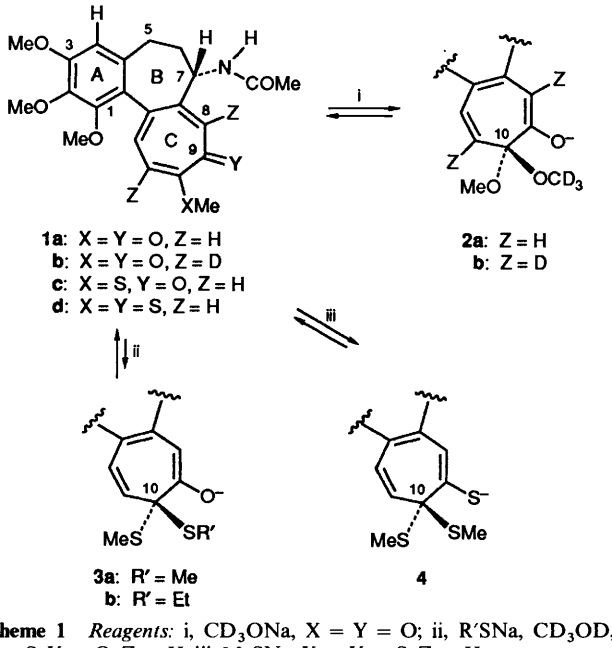
Marino Cavazza,[a] Carlo A. Veracini[a] and Francesco Pietra[b]
[a] *Dipartimento di Chimica e Chimica Industriale, Università di Pisa, 56126 Pisa, Italy*
[b] *Istituto di Chimica, Facoltà di Scienze MFN, Università di Trento, 38050 Povo-Trento, Italy*

^{1}H NMR spectroscopic monitoring in 28:1 $(CD_3)_2SO$–CD_3OD at room temp. shows that colchicine **1a** reacts extensively with equimolar $NaOCD_3$ giving reversibly the σ-adduct **2a**, *via* CD_3O^- addition at C-10, as proved by similar experiments with [8,11-$^2H_2$]colchicine **1b**, in which **2b** was obtained. Under similar conditions, dithiocolchicine **1d** also reacts extensively with equimolar NaSMe *via* MeS^- addition at C-10 to give adduct **4**. In contrast, isocolchicine **8** and equimolar $NaOCD_3$ give only very little σ-adduct (of alternative structure **9** or **10**) whereas in the case of thiocolchicine **1c** and equimolar NaSEt only broadening of ring-C ^{1}H NMR signals, most markedly of 11-H, was observed, indicating rapid exchange at C-10. It is concluded that, among a few analogies, the behaviour of colchicinoids toward bases differs sharply from that of troponoids.

We have extensively studied the reactions of troponoids with bases, where substitution or ring contraction occurs through the formation of reversible, covalent adducts.[1] Colchicine **1a**, an alkaloid of the meadow saffron *Colchicum autumnale*, possesses a 2-methoxycycloheptatrienone ring which undergoes nucleophilic reactions typical of 2-methoxytropone itself.[2] Therefore, we were interested to investigate whether the behaviour of colchicine is representative of the colchicinoids in general, and if σ-adducts are involved in their reaction with bases. The results of this investigation are reported here.

Results and Discussion

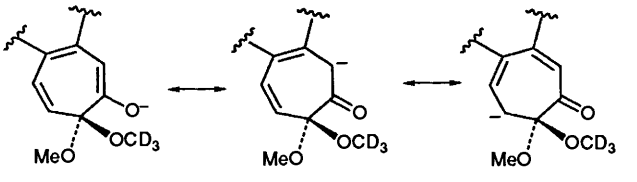
On addition of $NaOCD_3$, in slight molar excess, to colchicine **1a** in dried 28:1 $(CD_3)_2SO$–CD_3OD, ^{1}H NMR signals appeared which can be attributed to 4-, 8-, 11- and 12-H of adduct **2a** * (Table 1 and Scheme 1), besides signals for both the



Scheme 1 *Reagents:* i, CD_3ONa, X = Y = O; ii, R'SNa, CD_3OD, X = S, Y = O, Z = H; iii, MeSNa, X = Y = S, Z = H

ring-C protons and 4-H of unchanged **1a** (*ca.* 30%); the remaining ^{1}H NMR signals for both **2a** and unchanged **1a** were buried together. On neutralization of this mixture, the NMR signals for adduct **2a** immediately disappeared while those for colchicine reappeared, at 100% of their original level, when deuterium incorporation at the nitrogen atom and OCD_3–OCH_3 exchange are taken into account.†

The ^{1}H NMR spectral assignment in Table 1 for adduct **2a** takes into account the upfield shift expected for negative charge delocalization at C-8 and C-11, as represented by the canonical forms in Scheme 2. This attribution parallels that for



2a

Scheme 2

adduct **6** from 2-methoxytropone **5a** and methoxide (Scheme 3), where the protons were unambiguously assigned through deuteriation.[3] This points to a somewhat flattened seven-membered ring in adduct **2a**, in spite of tetrahedral C-10, in analogy with adduct **6**.[3]

The above assignment of adduct **2a** was confirmed by deuteriation. Thus, [8,11-$^2H_2$]-colchicine **1b**, prepared as described below (Scheme 4), was found to react with CD_3ONa,

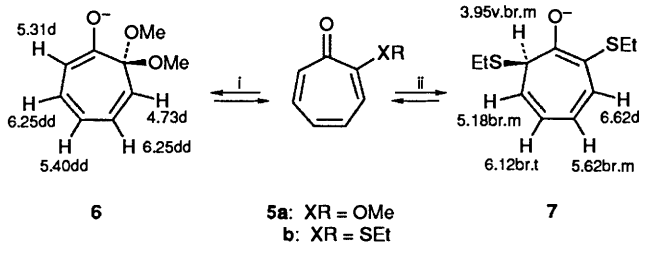
* The configuration at C-10 can be reversed.

† When **1a** and $NaOCD_3$ were mixed together with special care to avoid traces of moisture, we observed, besides the signals mentioned earlier for adduct **2a**, two sets of signals identical to the 8-H s and the 11-H/12-H AB system of colchicine **1a** (Table 1), each was separated from the companion signal by 2.4 Hz and integrated with respect to it for *ca.* 3:1. On neutralization of this mixture, all these NMR signals disappeared while the signals for colchicine were raised to 100% of their original level. This doubling of the NMR signals might result from the presence, besides residual colchicine, of a colchicine analogue, deriving from interaction of methoxide with the acetylamino group of colchicine. Possibly, it is the higher activity of methoxide under anhydrous conditions that triggers this phenomenon. An alternative rationalization in terms of σ-adducts resulting from nucleophilic attack by methoxide on colchicine at either C-1 or C-3—two ring positions which would feel the electron-withdrawing effect of the cycloheptatrienone ring should it be coplanar with the benzene ring—is ruled out by the lack of ^{1}H NMR-detectable OCD_3–OCH_3 exchange at the benzene ring. The presence of more than one chiral species is also suggested by a complex dichroic spectrum, showing two negative Cotton effects, at longer (378 nm) and shorter wavelength (327 nm) than the original single negative Cotton effect of colchicine (340 nm).

Table 1 ^{1}H NMR spectroscopic data for colchicine **1a**, [8,11-$^2H_2$]-colchicine **1b**, thiocolchicine **1c**, dithiocolchicine **1d**, isocolchicine **8**, and their adducts **2a**, **2b**, **4**, and **9** or **10** with alkoxides or thiolates [in 28:1 $(CD_3)_2SO$–CD_3OD, unless otherwise stated[a]]

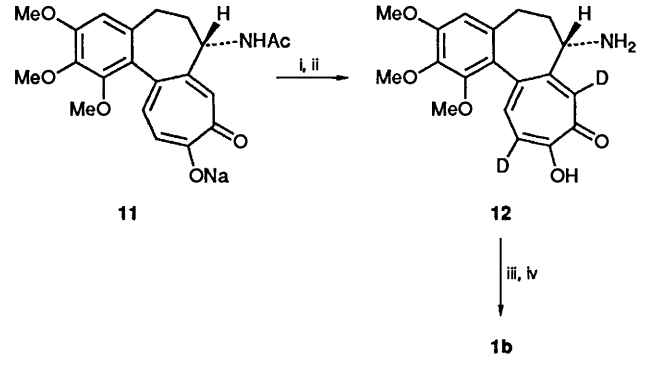
	δ_H								
Proton	**1a**[b]	**1b**[b]	**1c**[b]	**1d**[b]	**2a**	**2b**	**4**	**8**[b]	**9** or **10**[c]
1-MeO	3.63 s	3.63 s	3.74 s[d]	3.60 s[e]				3.50 s	
2-MeO	3.95 s	3.95 s	4.05 s[d]	3.90 s[e]				3.75 s	
3-MeO	3.90 s	3.90 s	4.00 s[d]	3.85 s[e]				3.82 s	
4-H	6.89 s	6.89 s	7.0 s	6.53 s	6.66 s	6.65 s	6.65(s)	6.78 s	6.64 s
5-H	2.7 m	2.7 m	2.8 m	2.6 m				2.53 m	
6-H	2.3, 2.1, 1.9 m	2.3, 2.1, 1.9 m	2.4, 2.2, 2.0 m	2.3, 2.1, 1.9 m				2.2–2.0 m	
7-H	4.44 m	4.44 m	4.54 m	4.65 m				4.32 m	
N*H*Ac	8.69 d, 7.4	8.69 d, 7.4	8.82 d, 6.9	8.68 d, 7.5				8.60 d, 6.9	
NH*Ac*	1.96 s	1.96 s	2.06 s	1.8 s				1.85 s	
8-H	7.25 s	—	7.24 s	8.4 s	5.47 s	—	5.12(s)	7.2 s	6.47 s
9-MeX								3.86 s	
10-MeX	3.99 s	3.99 s	2.62 s	2.40 s					
11-H	7.14 B	—	7.36 B	7.14 B	4.84 B	—	5.8(B)	6.91 B	5.17 B
	AB, 10.8		AB, 10.8	AB, 12	AB, 10.5		AB, 12.8	AB, 12.6	AB, 12.0
12-H	7.23 A	7.22 br s	7.47 A	7.44 A	6.45 A	6.46 br s	6.4(A)	7.20 A	6.34 A

[a] *J* values are given in Hz after the symbol for the signal pattern. [b] In neat $(CD_3)_2SO$. [c] Uncertain structural assignment. [d] These signals can be interchanged. [e] These signals can be interchanged.



6 **5a**: XR = OMe **7**
b: XR = SEt

Scheme 3 *Reagents:* i, NaOMe, XR = OMe; ii, NaSEt, XR = SEt



11 **12** **1b**

Scheme 4 *Reagents:* i, D_2O; ii, H^+; iii, AcCl–pyridine; iv, CH_2N_2

under similar conditions to those given above for **1a**, to give a ^{1}H NMR spectrum (Table 1) where the signals assigned to 8- and 11-H for **2a** are missing, as expected for structure **2b** of the adduct.

That the similarity between colchicinoids and troponoids with respect to base addition is limited to the above observations, was shown by mixing thiocolchicine **1c**[4] with either sodium ethanethiolate or sodium methanethiolate under conditions similar to the above experiments with **1a** and $NaOCD_3$. On addition of either one of the above thiolates to **1c**, a selective broadening of the ring-C hydrogens of **1c** was observed, most markedly 11-H, without any observable upfield shift. This can be rationalized in terms of an equilibrium between **1c** and adduct **3a** or **3b**,* in only trace amounts and with a frequency of exchange at C-10 which is on the NMR timescale. This is represented in Scheme 1 with unequal arrows between **1c** and **3**. On neutralization of the mixture resulting from **1c** and NaSMe, unchanged **1c** was obtained quantitatively, whereas in the case of the mixture from **1c** and NaSEt a *ca.* 1:1 mixture of ethylthiocolchicine[5] and **1c** was obtained, as expected from rapid exchange at C-10. Results similar to those above for **1c** and NaSMe were obtained with **1c** and NaOMe, except for the additional complexity introduced by SMe–OMe exchange at C-10, which made colchicine available and thus also led to the non-deuteriated analogue of **2a**.

That the equilibrium of the reaction of **1c** with R'SNa to give **3** is strongly displaced toward **1c** (Scheme 1) conforms to a generally observed thermodynamic instability of gem-dithio σ-anionic adducts, both in the troponoidal[1,6] and the benzenoid series.[7] However, on the basis of our knowledge of 2-alkylthiotroponoids,[1,6] where adducts at the unsubstituted C-7 (such as **7** from **5b** and NaSEt) are smoothly formed,[6] attack by the thiolate at C-8 of **1c** was expected, contrary to what has been observed.

In the hypothesis that the above failure to form a **7**-type adduct with thiocolchicine **1c** is due to steric interference among the groups at the tetrahedral reaction centre and neighbouring groups, NaSEt was added to *N*-deacetylthiocolchicine[8] in dried $(CD_3)_2SO$; no change in the spectrum of this colchicinoid was observed, however. This may mean that either deacetylation failed to remove steric hindrance to attack at C-8, or that the origin of lack of affinity by the thiolate for C-8 has to be found elsewhere.

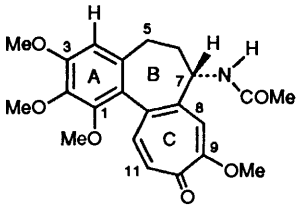
Lack of thiolate addition at C-8 is a property of dithiocolchicine **1d**,[9] too, but in this case the *gem*-dithio adduct **4** was formed smoothly (Scheme 1). This is noteworthy since stabilization of *gem*-dithio σ-anionic adducts has always proved to be a challenging problem; in the benzenoid series this was solved by spirocyclization,[10] which failed *per se* to afford stability in the troponoidal series.[11] With troponoids, it was only a conjugated nitro group at C-5 that could somewhat prolong the life of gem-dithio σ-anionic adducts.†[11]

In contrast to the behaviour of colchicine, isocolchicine (prepared by the new procedure described in the Experimental

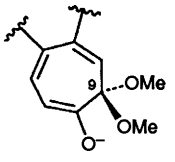
* The configuration at C-10 can be reversed.

† It is curious that modification of the NMR spectra of colchicine **1a** and the colchicinoids **1c** and **1d**, mostly at C-10 and neighbouring areas, on base addition parallels prevalent broadening of the ^{13}C NMR resonance of the C-10 methoxy group of colchicine in the interaction with tubulin.[12]

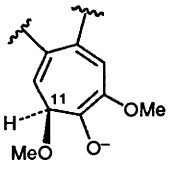
Section) shows poor affinity for $NaOCD_3$. Thus, when treated with $NaOCD_3$, under conditions identical with those above for colchicine, isocolchicine **8** was left largely unchanged; only at higher sensitivity a series of weak, sharp signals (Table 1, **9** or **10**) could be detected alongside broad weak signals centred at



8



9



10

δ 6.0 and 5.3. On neutralization of this mixture, all these weak signals disappeared, leaving only the NMR signals for isocolchicine. This pattern of sharp, weak ^1H NMR signals (Table 1, **9** or **10**) differs from that pertaining to adduct **2a** (Table 1) insofar as only one signal (δ 5.17) is shifted to higher field. We offer a tentative rationalization in terms of two alternative structures (**9** or **10**) for σ-anionic adducts where the seven-membered ring must be markedly bent so as to inhibit negative-charge dispersal, except within the enolate group C-11–C-10–O^-; this is at variance with charge distribution in the σ-adducts in Schemes 1–3, and previous σ-adducts from troponoids, which implies flattening of the seven-membered ring in spite of the sp^3 carbon in the seven-membered ring.*[1] In one of the proposed anomalous structures **9**, where inhibition of negative-charge dispersal at C-8 allows for shielding of only the enolate hydrogen at C-11, which may thus resonate at higher field than the other hydrogens. With the alternative structure **10** there are no enolate hydrogens and negative-charge dispersal at C-12 cannot occur in the bent ring; thus the hydrogen at C-11 may be selectively shifted to higher field because of the sp^3 nature of this carbon.

The results presented here show that the colchicinoids have limited resemblance to the troponoids as far as reactions with bases are concerned. In fact, while colchicine closely resembles 2-methoxytropone in this respect, isocolchicine does not; in a similar vein, thiocolchicine does not resemble 2-ethylthiotropone.

Experimental

Chromatography and Spectroscopy.—Preparative TLC was performed using 2 mm thick Merck Kieselgel 60 F_{254} plates. Reversed-phase HPLC was performed on Merck RP18 columns, 250 × 4.6 mm. NMR spectra were obtained using a Varian VXR 300 spectrometer at 299.94 MHz (^1H); δ values are reported with respect to TMS (0 ppm). UV spectra were obtained using a Perkin-Elmer Hitachi 200 spectrophotometer.

* With the only exception of the adduct of MeO^- at C-2 of 2-methoxy-3-nitro-4,5-benzotropone, where the seven-membered ring must be markedly non-planar.[13]

Materials.—$(CD_3)_2SO$ and CD_3OD were dried by distillation from $CaCl_2$ under dry N_2 and were stored over activated 3 Å molecular sieves. Natural, laevorotatory colchicine, the NMR spectrum of which in $CDCl_3$ has been assigned,[14] was purchased from Aldrich, whereas thiocolchicine and dithiocolchicine were obtained from Dr. A. Brossi. *N*-Deacetylthiocolchicine was prepared from thiocolchicine according to reported procedures.[8]

Reaction Between Colchicine and Sodium Methoxide for Spectral Observations.—A 1.5 mol dm^{-3} solution of $NaOCD_3$ in CD_3OD (25 mm^3) was added to a 0.045 mol dm^{-3} solution of **1a** (0.7 cm^3) in $(CD_3)_2SO$, under anhydrous conditions in an atmosphere of N_2. When the NMR observation had been completed (*ca.* 20 min), this mixture was neutralized with 6 mol dm^{-3} DCl in D_2O. In parallel, the dichroic spectrum was obtained at similar concentrations with a short optical-path cell (0.2 mm) for both **1a** ($\varepsilon_{max}(340) = -8.22$ mol dm^3 cm^{-1}) and the mixture following NaOMe addition, which consisted of two negative Cotton effects of comparable intensity at longer (λ_{max}/nm 378) and shorter (λ_{max}/nm 327) wavelength.

Preparation of [8,11-$^2H_2$]*Colchicine* **1b**.—The sodium salt of colchiceine **11** [obtained from the mild acid hydrolysis of **1a** to give colchiceine[15] (0.11 g, 0.28 mmol) followed by the addition of equimolar NaOH and drying] was heated in D_2O in a sealed ampoule at 120–140 °C for 9 d. The mixture was cooled, freed of tarry material by filtration, and acidified with dilute HCl whereby [8,11-$^2H_2$]-*N*-deacetylcolchiceine **12** was produced (0.98 g, 98%); $\delta_H(CDCl_3)$ 3.62 (3 H, s, 1-MeO), 3.93 (3 H, s, 2-MeO), 3.88 (3 H, s, 2-MeO), 6.54 (1 H, s, 4-H), 2.52 (2 H, m, 5-H), 2.32–2.05 (2 H, m, 6-H), 4.65 (1 H, m, 7-H) and 7.57 (1 H, s, 12-H); *m/z* 345 ($M^{\cdot+}$, 4%) and 314 ($[M - OMe]^+$, 32%). [8,11-$^2H_2$]-*N*-Deacetylcolchiceine **12** (0.98 g, 0.28 mmol) in dry pyridine (0.6 cm^3) was added to AcCl (0.1 cm^3) at room temp.; after one night the mixture was added to water and extracted with $CHCl_3$. The residue of evaporation was added to 9:1 Et_2O–MeOH (10 cm^3) and treated with an excess of CH_2N_2 in Et_2O for 6 h. The mixture was evaporated and the residue was subjected to TLC with 96:4 $CHCl_3$–MeOH; the R_f 0.2 band was collected to give **1b** (0.030 g, 26%). Spectral data are given in Table 1.

Reactions Between the Remaining Colchicinoids of Schemes 1 *and* 2, *or Isocolchicine* **8**, *with the Indicated Alkoxides or Thiolates for Spectral Observations.*—The general methodology and conditions are the same as those indicated above for colchicine and NaOMe, while the results are described in the text. Thiocolchicine **1c**[4,16] and dithiocolchicine **1d**[9] were obtained from Dr. A. Brossi, and isocolchicine **8**[17] was prepared according to the new procedure described below, whereby are circumvented (a) tedious and inefficient separation from colchicine, which is obtained together with isocolchicine in the methylation of colchiceine with diazomethane,[17] and (b) difficult separation of isocolchicine (obtained in trace amounts from Dr. A. Brossi) from colchicine by reversed-phase HPLC.

Synthesis of Isocolchicine.—Colchiceine (0.2 g, 0.52 mmol) in dry pyridine (0.3 cm^3) was added to mesyl chloride in 1.2 molar excess under N_2. The mixture was stirred at room temp. for 6 h, then added to water (10 cm^3), and then extracted with CH_2Cl_2 (3 × 5 cm^3). The organic layer was washed with 10% $CuSO_4$ and then water and then dried (Na_2SO_4). The solvent was evaporated at reduced pressure and the yellow solid residue (0.23 g) was subjected to preparative TLC with 95:5 AcOEt–EtOH to give as a yellow solid, 9-demethoxy-9-mesylisocolchicine, R_f 0.49 (0.067 g, 30%), $\lambda_{max}(CH_3CN)$/nm 355 (ε/dm^3 mol^{-1} cm^{-1} 8900), 321 (8900) and 232 (11 250); $\delta_H(CDCl_3)$ 3.70,

3.90, 3.91 (3 × 3·H, s, for the 3 MeO), 6.57 (s, 4-H), 2.53 (2 H, m, 5-H), 2.3–2.0 (2 H, series of m, 6-H), 4.53 (1 H, td, *J* 12.6, 6.2, 7-H), 6.0 (1 H, d, *J* 6.2, NH), 2.03 (3 H, s, Ac), 7.6 (1 H, s, 8-H), 3.45 (3 H, s, SMe), 7.47 and 7.17 [2 H, AB system, *J*(AB) 13.0, 11-H and 12-H]; *m/z* 384 ($[M - CH_3SO_2]^{\bullet+}$, 11%). The R_f 0.23 band gave yellow, solid 10-demethoxy-10-mesylcolchicine (0.048 g, 21%); $\delta_H(CDCl_3)$ 3.66, 3.89, 3.92 (3 × 3 H, s, for the 3 MeO), 6.51 (1 H, s, 4-H), 2.57 (2 H, m, 5-H), 2.4–2.2 (2 H, series of m, 6-H), 4.6 (1 H, m, 7-H), 5.85 (1 H, d, *J* 7.0, NH), 2.0 (3 H, s, Ac), 7.37 (1 H, s, 8-H), 3.51 (3 H, s, SMe), 7.46 and 7.19 [2 H, AB system, *J*(AB) 10.3, 11-H and 12-H]; *m/z* 463.1 ($M^{\bullet+}$, 1.3%), 384 ($[M - CH_3SO_2]^{\bullet+}$, 5%). To 9-demethoxy-9-mesylisocolchicine (0.056 g, 0.12 mmol) in dry $(CD_3)_2SO$ (1 cm^3) was added NaOMe (0.3 mmol in 0.2 cm^3 of dry MeOH) under N_2 at room temp. After 20 min the clear homogeneous solution was added to H_2O (10 cm^3) and then extracted with $CHCl_3$. The organic extract was dried (Na_2SO_4) and then evaporated at reduced pressure to give a solid residue which was subjected to TLC with 85:15 AcOEt–EtOH. The R_f 0.13 band gave isocolchicine **8** (10 mg, 21%).

Reaction of Thiocolchicine **1c** *with NaSEt for Preparative Purposes.*—Working as described above for spectroscopic purposes, albeit at a ten-fold higher scale, the mixture prepared from the title reagents was neutralized with dilute aq. HCl and then repeatedly extracted with $CHCl_3$. The organic phase was dried (Na_2SO_4), evaporated, and the semi-solid yellow residue was subjected to reversed-phase HPLC with CH_3CN–H_2O 4:6, 1 cm^3 min^{-1}, to give thiocolchicine,[16] t_R 7.1 min, and ethylthiocolchicine,[5] t_R 10 min, in *ca.* 1:1 ratio and practically quantitative overall yield.

Acknowledgements

We thank Dr. A. Brossi for samples of thio- and dithiocolchicine, Dr. M. Zandomeneghi for the CD spectrum, and Mr. A. Sterni for recording the mass spectra. This work was financially supported by MURST (*Progetti* 40% and 60%) and, in part, CNR, Roma.

References

1 F. Pietra, *Acc. Chem. Res.*, 1979, **12**, 132.
2 H.-G. Capraro and A. Brossi, in *The Alkaloids*, ed. A. Brossi, Academic Press, New York, 1984, vol. XXIII, p. 1–70.
3 G. Biggi, C. A. Veracini and F. Pietra, *J. Chem. Soc., Chem. Commun.*, 1973, 523.
4 P. Lincoln, J. Nordh, J. Deinum, A. Ångström and B. Nordén, *Biochemistry*, 1991, **30**, 1179.
5 G. T. Shiau, K. K. De and R. E. Harmon, *J. Pharm. Sci.*, 1975, **64**, 646.
6 C. A. Veracini and F. Pietra, *J. Chem. Soc., Chem. Commun.*, 1974, 623.
7 G. Biggi and F. Pietra, *J. Chem. Soc., Chem. Commun.*, 1973, 229.
8 L. Velluz and G. Muller, *Bull. Soc. Chi. Fr.*, 1954, 1072.
9 A. Muzaffar, A. Brossi, C. M. Lin and E. Hamel, *J. Med. Chem.*, 1990, **33**, 567.
10 E. Farina, C. A. Veracini and F. Pietra, *J. Chem. Soc., Chem. Commun.*, 1974, 672.
11 M. Cavazza, C. A. Veracini, G. Morganti and F. Pietra, *J. Chem. Soc., Chem. Commun.*, 1978, 167.
12 S. B. Hastie and R. P. Rava, *J. Am. Chem. Soc.*, 1989, **111**, 6993; I. Ringel and H. Sternlicht, *Biochemistry*, 1984, **23**, 5644.
13 V. Farina, M. Cavazza, R. Cabrino, C. A. Veracini and F. Pietra, *Tetrahedron Lett.*, 1976, 1319.
14 D. Meksuriyen, L.-J. Lin, G. A. Cordell, S. Mukhopadhyay and S. K. Banerjee, *J. Nat. Prod.*, 1988, **51**, 88; H.-G. Capraro and A. Brossi, *Helv. Chim. Acta*, 1979, **62**, 965.
15 G. A. Nichols and D. S. Tarbell, *J. Am. Chem. Soc.*, 1953, **75**, 1104.
16 L. Velluz and G. Muller, *Bull. Soc. Chim. Fr.*, 1954, 755.
17 M. Sorkin, *Helv. Chim. Acta*, 1946, **29**, 246.

Paper 2/03350J
Received 25*th June* 1992
Accepted 17*th August* 1992