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Synthesis of Cephalotaxine Esters and Correlation of Their Structures with Antitumor Activity

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Twenty-two new esters of natural (–)-cephalotaxine with synthetic acids possessing widely divergent structural features have been synthesized. Murine antitumor (P388 system) test data reveal that the methyl itaconate (**7a**) and trichloroethyl carbonate (**27**) esters of cephalotaxine are the most active of this group; this activity is less than that of harringtonine and other naturally occurring cephalotaxine esters. Other synthetic esters exhibiting activity are methyl cephalotaxylfumarate (**4**) and the trichloroethyl carbonate of cephalotaxyl-L-mandelate (**21**). The specificity of this experimental tumor system apparently requires esters of (–)-cephalotaxine for tumor inhibition because methyl cephalotaxylitaconate (**7b**) prepared from the synthetic (+) enantiomer of cephalotaxine is inactive.

Investigation of alkaloids isolated from *Cephalotaxus harringtonia* extracts demonstrated that several natural esters of the parent alkaloid, cephalotaxine (**1**),[1-4] exhibit significant activity against experimental leukemia systems.[3] However, plant material from which to extract these esters is in critically short supply. This shortage, coupled with the fact that cephalotaxine has now been synthesized,[5] has stimulated efforts to convert cephalotaxine (which possesses no activity in the unesterified form) to some of the active, naturally occurring esters. However, very unfavorable steric (and perhaps electronic) interactions at the reaction sites of both the cephalotaxine and the acyl moiety preclude direct esterification of these acids with cephalotaxine as a route to the active natural esters.[4,6a] Conversion of cephalotaxine (**1**) to its active esters has been achieved in the case of deoxyharringtonine[7a,b] (**10a**) and harringtonine;[7c] all three syntheses were carried out by indirect routes. Although significant, our partial synthesis of **10a** did not appear to be sufficiently high yielding to provide a practical source of active esters. Yield data for the Chinese worker's syntheses are not available at this time.

Chemical aspects of these active alkaloid esters have recently been supplemented by results of biological investigations concerned with their mode of action.[8] Huang[8c] states that harringtonine is the only small molecule thus far reported that penetrates animal cell membranes and selectively inhibits initiation of protein synthesis without affecting chain elongation.

Our current research was designed to further delineate structure–antitumor activity relationships[6] (beyond those provided by the natural esters) by preparing, as shown in Table I, various types of cephalotaxine esters (Chart I) not subject to the severe steric requirements mentioned earlier. In view of the plant material shortage, we also hoped to discover new, easily prepared esters with tumor inhibitory properties comparable to those of the natural esters. The esters to be synthesized (shown in Chart I) were selected to include acyl groupings with widely diverse structural features and also to include series of α,β-unsaturated, dicarboxylic, and aromatic esters.

Chemistry. In general, acylation reactions between cephalotaxine and the acylating agents produced highest yields if allowed to proceed at room temperature for an extended time period. Refluxing the reactants gave a greater mixture of products and greatly enhanced the tarry nature of the mixture. An interesting feature of the acylations that has not been explored concerns the four syntheses involving inverse addition of only a slight excess of acid chloride to alcohol (compounds **2**, **25**, **26**, and **27** in Table I). Two of these reactions yielded products (**2** and **27**) that were pure as isolated but this result could also be due in part to the fact that these particular two esters were extracted from pH 7.0 phosphate buffer instead of Na_2CO_3 solution. Buffer was used to provide conditions as mild as possible for these structures whose stabilities were unknown at the time. The other two products of inverse addition, while not pure as isolated, were easier to purify than those produced by the normal addition procedure. Our earlier work[6] indicated that an excess of at least 50% of acid chloride was desirable, if not mandatory, for good acylation yields, but results with the chloro-

Table I. Synthetic and Analytical Data of Cephalotaxine Esters

Compd	Synth methods[a] (isolated yield, %)[b]	Molar ratio of acylating agent/ cephalotaxine	Purificn methods[c,d]	Formula	Mp, °C	Analyses	High-resolution MS M+ calcd	High-resolution MS M+ obsd
2	A, II (71)	1.10	8	$C_{22}H_{25}NO_7$			415.163	415.162
3	C, I (49)	2.06	2, 7	$C_{23}H_{27}NO_7$			429.179	429.179
4	D, I (27)	2.56	2, 4	$C_{23}H_{25}NO_7$			427.163	427.162
5[e]			1	$C_{24}H_{29}NO_7$			443.194	443.194
6	C, I (39)	2.82	7, 1	$C_{24}H_{27}NO_7$			441.179	441.179
7a	C, I (83)	2.20	2	$C_{24}H_{27}NO_7$			441.179	441.179
7b	C, I (64)	3.73	2	$C_{24}H_{27}NO_7$			441.179	441.179
8, 9, 10a,b	Synthesized and reported previously[f]							
11	F, I (44)	1.50	1, 6a	$C_{25}H_{27}NO_7$	174-176	C, H, N	453.179	453.179
12	D, I (20)	2.08	5, 7,[g] 6b	$C_{19}H_{23}NO_6S$	168 dec		h	h
13	D, I (45)	1.92	1, 6a	$C_{20}H_{22}NO_5Cl$	140-141	C, H, N	393.116	393.116
14	A, I (14)	3.00	5, 1	$C_{21}H_{23}NO_5$			369.158	369.156
15	A, I (56)	3.00	1	$C_{22}H_{25}NO_5$			383.173	383.172
16	Synthesized and reported previously[f]							
17	B, I (36)	2.00	1, 4	$C_{24}H_{27}NO_5$			409.189	409.188
18	F, I (71)	2.10	1, 5, 6c	$C_{25}H_{25}NO_8$	130-132	C, H, N	467.158	467.156
19	E, I (37)	2.00	1	$C_{24}H_{24}N_2O_5$			420.169	420.169
20	A, I (64)	4.06	5, 1, 7, 6a	$C_{25}H_{25}NO_5$	170-171	C, H, N	419.173	419.175
21	C, I (91)	1.50	1	$C_{29}H_{28}NO_8Cl_3$			i	i
22[e]			1	$C_{26}H_{27}NO_6$			449.184	449.183
23	E, I (43)	2.07	5, 1, 7, 6a	$C_{27}H_{27}NO_5$	174-175	C, H, N	445.189	445.188
24	B, I (45)	2.50	3, 6a	$C_{27}H_{26}N_2O_7$	212-214	C, H, N	490.174	490.171
25	A, II (72)	1.12	1	$C_{21}H_{25}NO_6$			387.168	387.167
26	A, II (27)	1.07	5	$C_{26}H_{27}NO_6$			449.184	449.182
27	A, II (96)	1.10	8	$C_{21}H_{22}NO_6Cl_3$			491.048	491.047

[a] Synthetic methods: A, acid chloride used as obtained from commercial source; B, acid or half-ester from commercial source converted to acid chloride with oxalyl chloride; C, same as B, except acid or half-ester was first prepared by chemical modification of commercially available precursor (see Experimental Section); D, commercially available anhydride used; E, commercially available acid converted to acid chloride by 2-h reflux with thionyl chloride; F, same as E, except acid or half-ester was first prepared by chemical modification of commercially available precursor (see Experimental Section); I, cephalotaxine added to large excess of acid chloride or anhydride; II, acid chloride in 10% excess added slowly to cephalotaxine. [b] Based on cephalotaxine; not optimized. [c] Purification methods: 1, chromatography on neutral alumina (Woelm, grade 3), 1 × 10 cm column, eluted with diethyl ether; 2, same as 1, except eluted with diethyl ether-CH_2Cl_2 (2:1); 3, same as 1, except eluted with benzene, and then appropriate fractions were combined and rechromatographed on a new, identical column with diethyl ether-CH_2Cl_2 (3:1); 4, chromatography on silica gel (Hi-Flosil, 60-200 mesh, Applied Science), 2.5 × 35 cm column, eluted with CH_2Cl_2; 5, same as 4, except successive elution with 100 ml of CH_2Cl_2, 200 ml of MeOH-CH_2Cl_2 (1.5:98.5), 250 ml of MeOH-CH_2Cl_2 (3:97), and 250 ml of MeOH-CH_2Cl_2 (5:95); 6, crystallization (a) from diethyl ether by slow evaporation at room temperature, (b) from CH_2Cl_2 by dropwise addition of acetone at room temperature, and (c) from diethyl ether-ethyl acetate (1:1) at −20 °C; 7, preparative TLC; 8, ester pure as isolated. [d] Procedures applied in sequence listed. [e] See Experimental Section. [f] See ref 4 (for 8 and 9), 7a (for 10a,b), and 6a (for 16). [g] Preparative TLC solvent was $CHCl_3$-MeOH-H_2O (60:35:5). [h] Satisfactory high-resolution mass spectrum not obtained. [i] M^+ − 191 ($-C_3H_2O_3Cl_3$ group); calcd 432.179, obsd 432.181.

formates used in **25**, **26**, and **27** do not support this conclusion.

It was discovered that ester **4** is produced whether one begins with maleic anhydride or with methyl hydrogen fumarate acid chloride as the acylating agent. The NMR spectra are superimposable. All data in the tables applying to **4** were derived from the ester prepared with maleic anhydride, but because of double bond isomerization this ester is the fumarate as shown.

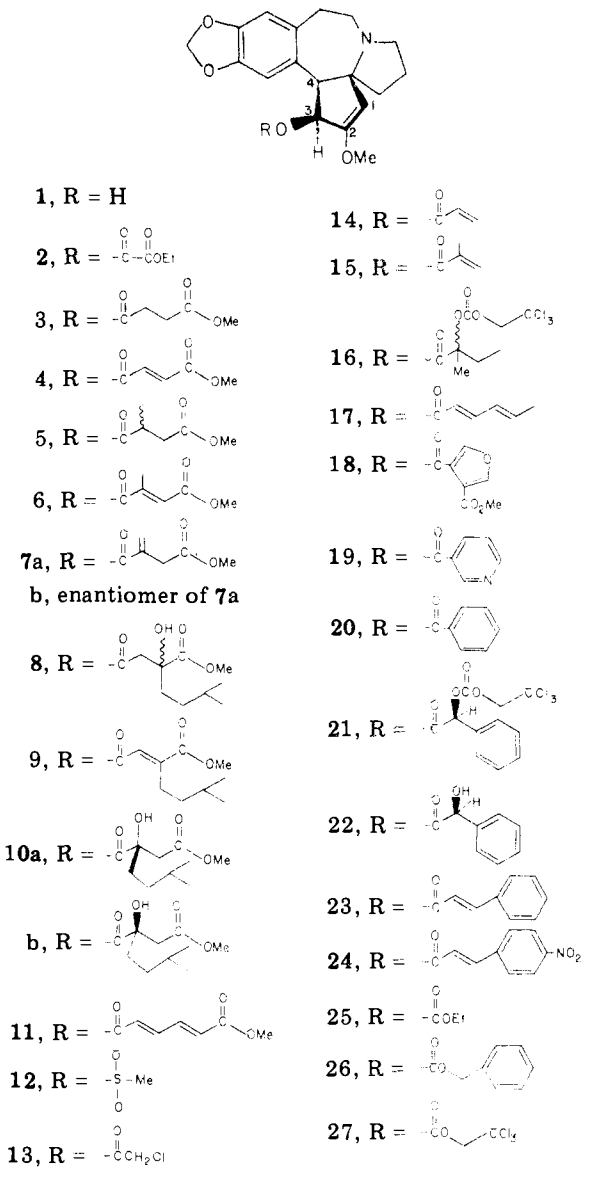
TLC analysis of the crude cephalotaxyl benzoate (**20**) preparation revealed an unusual component migrating immediately beneath and just touching the benzoate spot. The NMR spectrum of this material revealed that it contained precisely two benzoic acid residues and one cephalotaxine residue; certain chemical shifts clearly indicated that it was not simply a fortuitous 1:1 mixture of **20** and benzoic acid. A probe mass spectral analysis of this material below the temperature at which fragmentation of **20** is observed gave a preponderance of *m/e* 105 ($C_6H_5CO^+$) ions likely formed by rapid expulsion of the extra benzoic acid residue. We believe this residue was present either as a salt or some type of complex with **20**. Similar unusual components in comparable locations were observed during TLC analyses of the crude isolates of compounds **18** (furandioate), **23** (cinnamate), and **24** (*p*-nitrocinnamate).

Of the new esters we have prepared, only the methanesulfonate (**12**), the two acrylates (**14**,**15**), and the oxalate (**2**) gave evidence of instability. TLC analyses of **2**, **12**, and **15** after they had been standing at room temperature for various intervals showed additional spots; color darkening was apparent. Yield data for **12** and **14** probably also reflect instability.

Results and Discussion

Test data for all compounds showing any activity at all are reported in Table II. Dose levels other than those indicated were tested for many of the compounds but gave either toxic or inactive responses. Of the compounds listed, five exhibit significant and consistent antitumor activity against the experimental P388 lymphocytic leukemia system in mice. Test data for one of these actives (**10a**) are reported here only for comparison purposes and are comparable to those previously published.[3] Ester **10b**, which was isolated as an additional product in the partial synthesis of deoxyharringtonine (**10a**), differs from **10a** only in the absolute configuration of its acyl group which is *S* instead of *R*.[9] Although test data shown in Table II

Chart I



for **10b** are inconclusive, its lack of toxicity tends to support a conclusion that it is inactive since **10a** was toxic at doses above 5.9 mg/kg per injection.

Compounds **7a**, methyl cephalotaxylitaconate, and **27**, trichloroethyl cephalotaxylcarbonate, are also active. The itaconate shows consistent activity on the order of T/C = 130–200 over a dose range of about 40–365 mg/kg per injection. One 30-day cure was realized. Ester **7a** is toxic at the 400 mg/kg per injection level. Preliminary testing of **7a** in the L1210 system reveals no activity at doses ranging from 25 to 200 mg/kg per injection. The enantiomer of **7a**, ester **7b**, is methyl cephalotaxylitaconate prepared from synthetic (+)-cephalotaxine; it is inactive but is slightly toxic at 480 mg/kg per injection.

Trichloroethyl cephalotaxylcarbonate, **27**, has overall activity and effective dose levels very similar to **7a**; it gives T/C = 140–195 over the range of about 10–320 mg/kg per injection at which point it begins to be toxic. In contrast to **10a**, both **7a** and **27** are easily prepared from **1** in excellent yields.

Ester **4**, methyl cephalotaxylfumarate, seems to exhibit rather low, essentially unvarying activity from about 2 to 4 mg/kg per injection up to 80 mg/kg per injection. Further testing could prove it inactive.

The remaining active ester (**21**) is cephalotaxyl-L-mandelate having the hydroxyl group blocked with a trichloroethyl carbonate moiety; test data indicate it is active at the higher doses.

An interesting feature in these structure–activity correlations is the activity observed for two of the three esters incorporating a trichloroethyl carbonate moiety. This grouping is esterified directly with the cephalotaxine hydroxyl in ester **27**; in **21** it is esterified with the mandelate hydroxyl group and seems to impart activity to an otherwise inactive (**22**) ester. In addition, toxicity observed during administration of **22** (at doses above 80 mg/kg per injection) is not present with **21** at doses up to 320 mg/kg per injection. On the other hand, ester **16**, containing a trichloroethyl carbonate blocked hydroxyl, as well as its precursor with a free hydroxyl,[6a] is inactive. Both **16** and the precursor[6a] were prepared from a racemic

Table II. Biological Test Data[a] for Cephalotaxine Esters (P388 Lymphocytic Leukemia in Mice)

Compd	Vehicle[b]	Dose, mg/kg/inj[c]	Animal wt difference, T − C	T/C,[d] %
2	D	20	0.1	135
	D	20	−0.2	211
	D	13	−0.8	154
	T	20	−0.1	129
4	B	80	−0.7	145
	B	40	−0.9	134
	B	20	−1.0	125
	B	10	−1.2	136
	D	4.4	−1.5	147
	D	1.9	0.5	134
7a	D	365	−3.0	198[e]
	D	240	−1.4	169
	D	160	−0.1	183
	D	160	−1.1	167
	D	80	−0.1	173
	D	40	−1.3	135
9	C	20	0.9	131
10a	D	5.9	−0.9	184
	A	4	0.0	174
	A	2	0.8	126
10b	D	9	−2.2	131
	A	4	0.5	150
17	D	80	0.9	150
	D	40	−1.8	125
	D	20	0.9	130
21	A	320	−2.3	136
	A	160	1.2	154
	A	80	−1.0	138
27	D	320	−1.0	172
	D	160	−0.9	162
	D	160	−0.3	155
	D	80	−0.9	183
	D	80	−1.3	160
	D	40	−0.4	140
	D	40	0.9	183
	D	20	−2.7	128
	D	20	−1.5	160
	D	20	−1.0	195
	D	13	−0.5	138
	D	8.8	−0.3	170

[a] Assays were performed under the auspices of Drug Research and Development, National Cancer Institute; protocols are described in R. T. Geran, N. H. Greenburg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3, 1 (1972). [b] A = saline, B = water + alcohol + acetone, C = water + acetone, D = water + alcohol, T = saline + Tween 80. [c] One intraperitoneal injection per day for 9 days; DBA/2 mice. [d] T/C = mean survival time of test animals/mean survival time of control animals; 125% or above considered active. Unaccountable variations in T/C values among duplicate tests were sometimes observed; these may possibly be due to solubility properties of the esters in the vehicles used. [e] One 30-day cure was reported.

hydroxy acid. Other carbonate esters of cephalotaxine, the ethyl carbonate (25) and the benzyl carbonate (26), are inactive in this system and so is the remaining chloro derivative, the chloro acetate (13).

At one stage in the testing, aqueous 0.5% citric acid solution was used as the injection vehicle for esters 2 and 27. In contrast to erratic results obtained by using water + alcohol and saline + Tween 80 as vehicles (Table II), in which three separate tests of 20 mg/kg per injection doses gave T/C = 211, 129, and 135, 2 in citric acid vehicle was inactive. Similarly, 27, which demonstrated good tumor inhibitory action in water + alcohol vehicle, was completely without effect in citric acid solution.

Esters 9 and 17, in addition to those already mentioned, produced inconclusive data.

The antileukemic properties of 7a prompted us to synthesize other esters of 1 with α,β-unsaturated acyl groups (4, 14, 15, 17, 23, 24). Only 4 and 17 show any activity and the data for 17 are inconclusive. Increasing the similarity of an α,β-unsaturated ester to itaconate by incorporation of a second carboxyl grouping as in 6 and 11 fails to produce active compounds.

The stringent specificity requirements demonstrated by these unsaturated esters, and also by the trichloroethyl carbonates, are perhaps somewhat surprising in view of the results of similar treatment of maytansine. Kupchan et al.[10] exchanged the original amino acyl group ($-COCH-CH_3NCH_3COCH_3$) of maytansine with propionate, bromoacetate, and crotonate; all three esters exhibit antileukemic properties comparable to maytansine.

The unesterified alcohol moieties, on which certain potent tumor inhibitory esters are based, are inactive. These alcohols include cephalotaxine (1),[3] maytansinol,[10] and 12-hydroxydaphnetoxin.[11] Kupchan has suggested that perhaps certain antitumor esters require the ester grouping to act as a carrier moiety, e.g., in processes concerned with cell penetration or selective molecular complex formation.[11] However, in the case of cephalotaxine and its esters, cell penetration, at least, does not seem to present a problem because Huang[8c] reports that the same relative inhibition [cephalotaxine (1) compared with its esters] is observed whether the compounds are tested in intact reticulocytes or in cell-free reticulocyte lysates.

We have demonstrated that various esters having antileukemic activity can be synthesized from (-)-cephalotaxine and also that specificity for antitumor activity probably includes the absolute configuration of the cephalotaxine moiety. On the basis of results for our one example, esters of (+)-cephalotaxine will likely be inactive. All of the naturally occurring active esters contain a dicarboxylic acyl group and a tertiary hydroxyl group α to the carboxyl esterified with cephalotaxine. These structural features are now shown to be nonessential for tumor inhibition; however, the natural esters are 50–150 times more active (on a weight basis) than any of our synthetic esters. Perhaps one of the most interesting conclusions of this work is the apparent lack of activity–structure correlations within a series of similar esters such as the α,β-unsaturated esters.

Experimental Section

General. High-resolution mass spectral analyses were performed with a Nuclide 12-90G[12] spectrometer and low-resolution analyses with a Du Pont CEC 21-492-1 spectrometer. NMR data are reported (see paragraph at end of paper regarding supplementary material). IR spectra were recorded on 1% solutions in $CHCl_3$ with a Perkin-Elmer Model 137 spectrophotometer. Analytical TLC was done on Brinkmann precoated 0.25-mm silica gel F_{254} plates with 15:85 MeOH–$CHCl_3$ (for alkaloid esters) or with ether–hexane–acetic acid mixtures ranging from 10:88:2 to 50:48:2 (for acids, half-esters, and dicarboxylic acids). Spots were visualized by staining with I_2 vapor, by spraying with ethanolic bromothymol blue solution, or by charring with H_2SO_4–$K_2Cr_2O_7$ spray reagent.[13] Preparative TLC was done on 2-mm layers (also Brinkmann precoated) with MeOH–$CHCl_3$ (15:85) and using bromothymol blue or dichlorofluorescein for visualization. Anhydrous reagents, solvents, and solutions of reactants were prepared by drying for at least 4 h over type 3A or 4A molecular sieve. Extracts of aqueous systems were routinely dried with $MgSO_4$. All new compounds were amorphous solids[14] unless a melting point is given and each gave IR, NMR, and mass spectra consistent with its structure.

All purification steps were monitored by TLC and in most instances by IR. NMR and melting points were applied where appropriate.

Most of the (-) isomer of cephalotaxine used in our work was previously isolated at this laboratory from the natural source. A small portion of (-)-cephalotaxine and all of the (+) isomer used were synthetic.[15]

Modification of Acyl Moieties. (a) Methyl Hydrogen Succinate for 3. Dimethyl succinate was converted to methyl hydrogen succinate and purified by standard procedures: mp 56–58 °C (lit.[16] 58 °C); NMR δ 2.63 (s, 4, $-CH_2CH_2-$) and 3.67 (s, 3, $-OCH_3$).

(b) Methyl Hydrogen Fumarate for 4. Methyl hydrogen fumarate was prepared by partial esterification of fumaric acid as previously described.[7a] The half-ester was isolated by chromatography of the crude mixture on a 2.5 × 35 cm silica gel column using 300 ml of ethyl acetate–benzene (10:90) and 400 ml of ethyl acetate–benzene (20:80). The NMR spectrum of the pure half-ester showed δ 3.78 (s, 3, $-OCH_3$) and 6.86 and 7.20 (2 br s, 1 each, vinyl protons).

(c) Acid Chloride of 2-Methyl-3-carbomethoxyprop-2-enoic Acid for 6. Methyl hydrogen itaconate [see (d) below], 0.230 g, was hydrogenated in 5 ml of MeOH with 50 mg of 10% Pd/C; 1 molar equiv of H_2 was consumed. The resulting half-ester (0.214 g) was treated as described[17] for the synthesis of α-chloro acid chlorides. NMR analysis of the resultant mixture indicated that a major component was an acid chloride derived by dehydrohalogenation instead of by α-chlorination. This impure acid chloride was esterified with cephalotaxine to give 6.

(d) Methyl Hydrogen Itaconate for 7a,b. Methyl hydrogen itaconate was prepared from itaconic acid (248 g, 1.9 mol), MeOH (246 ml), and acetyl chloride (4 ml) as described by Baker et al.:[18] yield 115 g (42%); mp 66–68 °C (lit. mp 66–68 °C).

(e) Methyl Hydrogen Muconate for 11. Dimethyl muconate (7.35 g, 43 mmol) yielded 12 mmol (28%) of methyl hydrogen muconate when treated as described by Karrer and Stoll,[19] mp 164 °C (lit. mp 163 °C). Hot ethyl acetate was found to be a more workable crystallization medium than benzene in our hands.

(f) Methyl Hydrogen 3,4-Furandioate for 18. An 8.0-g sample (51 mmol) of 3,4-furandicarboxylic acid dissolved in 50 ml of 5% HCl in MeOH was stored over type 3A molecular sieve over a weekend. The concentrated residue was diluted with 4 vol of H_2O and the dimethyl ester was extracted with diethyl ether: yield 45 mmol (88%). This diester was dissolved in 100 ml of MeOH and 1.98 g (10% excess over 1 molar equiv) of NaOH in 25 ml of H_2O was added to the stirred solution over a 10-h period (phenophthalein indicator signaled when the base was all reacted). The resulting neutral solution was concentrated to one-fourth its original volume and was basified with 5% aqueous Na_2CO_3, and residual diester was extracted with diethyl ether. Extraction of the acidified (6 N HCl) aqueous phase with CH_2Cl_2 yielded 6.00 g of crude half-ester which was crystallized from a minimum quantity of hot ethyl acetate: yield 3.61 g (42%); mp 138–142 °C.

(g) 2-Trichloroethyl Carbonate Ester of L-Mandelic Acid for 21. L-Mandelic acid (66 mmol) was converted to the corresponding benzyl ester as previously described.[6a] By cooling the resulting benzene solution to about 10 °C, filtering, concentrating the liquor, and repeating the process twice, two fractions of 6.64 g (mp 97–99 °C) and 1.29 g (mp 102–105 °C) were obtained. Acidic materials were washed from the combined fractions (in $CHCl_3$) with 5% aqueous $NaHCO_3$. Evaporation of the solvent and recrystallization of the product from benzene yielded 28 mmol

(42%) of benzyl mandelate, mp 104–106 °C.

Treatment of benzyl mandelate (22 mmol) with Cl_3CCH_2OCOCl and recovery of the product (10.14 g) was accomplished as previously described.[6a,20] Purification was achieved with a 2.5 × 35 cm silica gel column using 2-g batches of crude product and eluting with benzene–petroleum ether—200 ml of 25:75, 200 ml of 35:65 and 45:55 until elution was complete. The benzyl ester carbonate crystallized from ether at 0 °C to give 15 mmol (68%), mp 96–97 °C. Hydrogenolysis of 6.13 g of this ester in THF with 100 mg of 10% Pd/C for each gram of ester until a molar equivalent of H_2 was consumed yielded 14.2 mmol (97%) of mandelic acid with its hydroxyl group esterified with a trichloroethyl carbonate group.

Synthesis. (a) Acid Chlorides. Typically, the acid or half-ester (5–25 mmol) was cooled to near 0 °C in an ice water bath and neat $(COCl)_2$, 2–10 ml, was added with stirring. This solution was allowed to come to room temperature and was stirred overnight (or a weekend). Excess reagent was removed in vacuo. When $SOCl_2$ was used, it was added neat to the acid or half-ester and the resulting solution was refluxed for 2 h. Excess reagent was removed in vacuo. Acid chlorides were generally used as recovered but were monitored by IR since residual $(COCl)_2$ or $SOCl_2$ reduces ester yields in the subsequent step through formation of *epi*-cephalotaxyl chloride.[6b]

(b) Cephalotaxyl Esters in General. In method I, the acid chloride or anhydride was dissolved in 5–20 ml of CH_2Cl_2 and cooled to near 0 °C in an ice water bath. Cephalotaxine (**1**, 2–10 mmol) and pyridine (threefold excess) in 15 ml of CH_2Cl_2 were added in a 10- to 15-min period from a dropping funnel. Stirring was continued while the solution warmed to room temperature and then overnight or a weekend; occasionally longer reaction times were needed.

Method II involved treating a cold CH_2Cl_2 solution of appropriate quantities (as in method I) of cephalotaxine and pyridine with a 10% excess of the acid chloride, also in CH_2Cl_2. It was added over a 2- to 3-h period from a dropping funnel. Stirring was continued as in method I.

The reaction mixtures from either method I to II were poured into 75–100 ml of 5% aqueous Na_2CO_3 solution (except for compounds **2** and **27**, where pH 7.0 phosphate buffer was used) and were extracted with three 75-ml portions of CH_2Cl_2. After the extracts had been washed twice with H_2O, the CH_2Cl_2 and residual pyridine were evaporated in vacuo to constant weight, and the crude esterification products (usually black and tarry oils) were subjected to one or more of the purification procedures (Table I).

(c) Methyl Cephalotaxyl Methylsuccinate (5). Compound **5** was prepared by hydrogenation of **7a** (3.3 mmol) in 10 ml of MeOH with 150 mg of 10% Pd/C. Under ambient conditions the cephalotaxine double bond was not reduced and 1 molar equiv of H_2 was consumed: yield of **5**, 72%.

(d) Cephalotaxyl L-Mandelate (22). Compound **21** (5.1 mmol) in 20 ml of glacial acetic acid was stirred at room temperature overnight with 3.4 g of activated zinc dust.[6a] A vigorous exothermic reaction began with addition of the zinc, and cooling in a cold water bath for 15 min was necessary. The reaction mixture was diluted with 20 ml of $CHCl_3$ and 20 ml of H_2O, and solid $NaHCO_3$ was added until the aqueous phase remained basic. Evaporation of the $CHCl_3$ phase yielded 3.9 mmol (76%) of cephalotaxyl L-mandelate (**22**).

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Supplementary Material Available: listing of NMR data (8 pages). Ordering information is given on any current masthead page.

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(12) The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.
(13) Dibasic acid and half-ester spots are best charred on precoated TLC plates by allowing the sprayed plate to stand for 10–15 min before placing it in a preheated oven at 170–180 °C for 3–6 min.
(14) Synthetic and natural esters of cephalotaxine are very resistant to crystallization (see ref 6b). Where melting points are reported, C, H, and N combustion analyses were done and were within ±0.4% of the calculated values. In cases where high-resolution mass spectrometry was used to verify molecular formulas, calculated and observed values agreed within ±0.003 mass units.
(15) Supplied through the courtesy of Dr. John D. Douros of the Drug Development Branch, National Cancer Institute. Racemic cephalotaxine was synthesized under National Cancer Institute Contract NIH 72-2002 by a modified Weinreb–Auerbach procedure (see ref 5a) and was resolved by fractional crystallization of the tartrate salts.
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