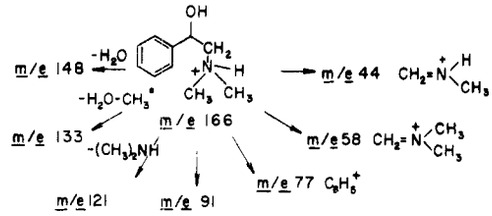
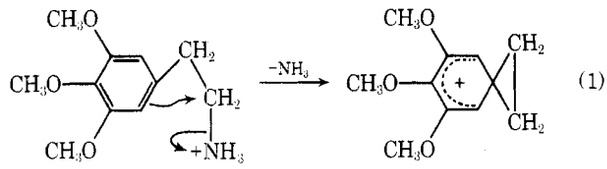
Scheme I. Major Collision-Induced Fragmentations of Protonated Ubine



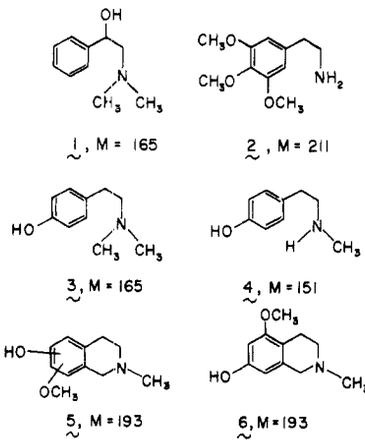
alkaloids of particular interest as well as in the characterization of new alkaloids. The occurrence of mescaline (2) in cactus extracts provides a case in point. The MIKE spectrum of protonated mescaline is characterized by an intense peak due to elimination of a fragment of 17 mass units. It is suggested that reaction 1 occurs as shown. The double analysis inherent



(1)

in the MIKES method, viz. analysis for the mass of the precursor ion as well as that of the fragments, makes the method highly specific. Mescaline could, therefore, be identified in *O. spinosior* simply on the basis of its molecular weight (211) and its fragmentation by loss of NH_3 from the protonated species. The MIKE spectrum of a peyote extract was studied to confirm this assignment. Further confirmation of the presence of mescaline in *O. spinosior* was obtained by comparing the MIKE spectra of the *fragment* ion due to NH_3 loss with that for the same ion in authentic mescaline hydrochloride and in peyote extracts.

The foregoing studies were made by chemical ionization. Electron impact ionization is also useful in this type of study although the resulting mass spectra can show extensive fragmentation which is a disadvantage in recognizing molecular ions. On the other hand, MIKE spectra obtained on molecular ions compare well with electron impact mass spectra of the pure compounds and this facilitates structural assignments. Hordenine (3) and *N*-methyltyramine (4) were identified in the *D. uberiformis* extract on the basis of the MIKE spectra shown in Table I. For comparison the electron impact mass spectra of the pure compounds are also shown.



1, M = 165 2, M = 211

3, M = 165 4, M = 151

5, M = 193 6, M = 193

Electron impact also showed the presence of a new alkaloid, molecular weight 193 in *D. uberiformis*. The MIKE spectrum of this alkaloid showed fragments formed by loss of 1, 15, 17, and 43 mass units. This was interpreted[6] as corresponding to the structure 5 in which the positions of the aryl substituents were not established. In independent work[4] the new alkaloid

Table I. Fragment Ions in Mass and MIKE Spectra

Hordenine (3)		*N*-Methyltyramine (4)	
Mass spectrum	MIKES	Mass spectrum	MIKES
121 (0.05)	(0.05)	149 (0.02)	
120 (0.04)	(0.01)	121 (0.03)	(0.01)
107 (0.09)	(0.05)	120 (0.05)	(0.01)
91 (0.08)	(0.01)	108 (0.06)	(0.13)
77 (0.15)	(0.02)	107 (0.11)	(0.07)
58 (1.00)	(1.00)	91 (0.03)	
		78 (0.02)	(0.01)
		77 (0.08)	(0.02)
		58 (0.42)	(0.01)
		44 (1.00)	(1.00)

uberine (6) was isolated from this plant and its structure was established by conventional methods.

In conclusion, the ion kinetic energy method of mixture analysis has been shown to be applicable to the identification and structural elucidation of alkaloids in crude plant extracts. The method represents an alternative to GC/MS and has comparable sensitivity and specificity. It may be particularly appropriate in studies of alkaloids and other involatile compounds for which gas chromatography is difficult.

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Registry No.—1, 34469-09-5; 2, 54-04-6; 3, 539-15-1; 4, 370-98-9; 5, 63715-57-1; 6, 63596-58-7.

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(6) The intense H· loss suggests the tetrahydroisoquinoline structure; loss of 43 (CH_3· + CO) is common to aryl methyl ethers and corresponds here also to retro Diels–Alder fragmentation.

Synthetic Studies on the Side Chains of Cephalotaxus Esters

Robert B. Bates,* Robert S. Cutler, and Richard M. Freeman

Department of Chemistry, University of Arizona, Tucson, Arizona 85721

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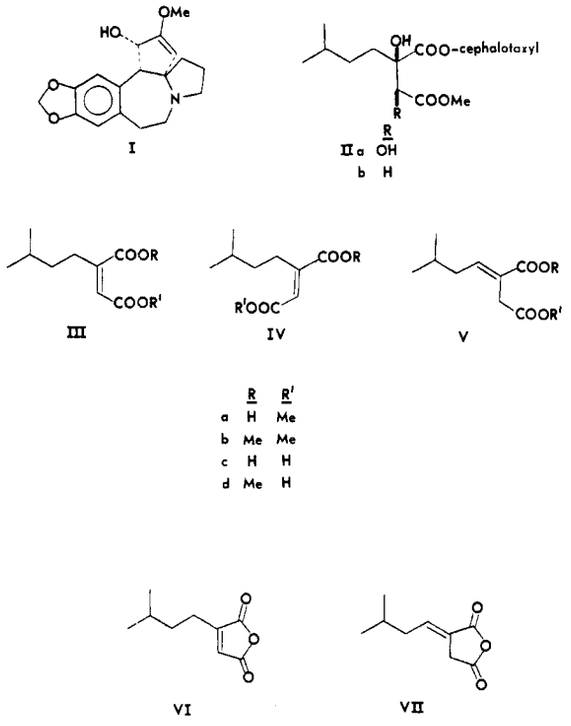
The naturally occurring antileukemic esters of cephalotaxine (I), e.g., isoharringtonine (IIa) and deoxyharringtonine (IIb), have proven to be formidable synthetic objectives due to steric problems in attaching the side chain to cephalotaxine;[1] in fact, the only reported success involved esterification with an α-keto acid (good yield) followed by addition to the keto group (very low yield) to give IIb.[2] It was also possible to esterify cephalotaxine (I) with two other acids with sp^2 hybridized α carbons: *p*-bromobenzoic acid[3] (80% yield) and half-ester IVa[4] (57% yield). We have independently been working on approaches to half-esters IIIa and Va in the hope that they might be sufficiently sterically unhindered to combine with cephalotaxine (I) to form esters which could be further transformed into IIa and IIb and wish to report more efficient routes to compounds in the III, IV, and V series than



those used earlier.[4,5] These new routes permit entry into each series in one step rather than several and in better yield.

Diisoamylcopper lithium added in the expected 1,4 manner[6-8] to dimethyl acetylenedicarboxylate, producing a mixture of IIIb and IVb. As expected,[6-8] the syn addition product IIIb predominates at −78 °C (89% in ether, 92% in THF), and on warming to 5 °C before quenching, the major product (60%) becomes IVb. Although diesters IIIb and IVb were readily separated by chromatography, the crude reaction mixture was suitable for the preparation of IIIa due to convergence at a later step.

Saponification of these esters was accompanied by shift of the double bond position to give Vc in excellent yield; any of the esters IIIb, IVb, and Vb with sodium methoxide gave an equilibrium mixture consisting almost exclusively of Vb. On acidic hydrolysis, however, IIIb and IVb gave the corresponding diacids IIIc and IVc smoothly without double bond shift. While IVc was readily purified by crystallization, IIIc was a viscous oil.

IIIc and Vc quantitatively produced the corresponding anhydrides, VI and VII, respectively, when refluxed in acetic anhydride. IVc, however, required heating with P_2O_5 at 200 °C to give VI.[5] With methanol at room temperature, anhydride VII reacted predominantly at the less hindered carbonyl,[10] giving desired half-ester IIIa (79%), along with lesser amounts of IIId (13%) and Va (8%).

A second good route to half-ester Va started with the Stobbe condensation of dimethyl succinate with 3-methylbutanal to the half-ester Vd (82%), which was hydrolyzed to diacid Vc (79%) and then converted to Va as above. The double bond could be partially shifted to the position between the carbonyl groups by equilibrating anhydride VII with anhydride VI using tri-*n*-butylamine at 125 °C; the equilibrium mixture contained only 35% of VI, however.

Our initial efforts to esterify cephalotaxine with half-esters IIIa and Va have been unsuccessful; apparently IIIa is sufficiently more sterically hindered than its stereoisomer IVa[4] to make esterification difficult.[11]

Experimental Section

Nuclear magnetic resonance (NMR) spectra of all compounds were measured on a Varian T-60 spectrometer. Melting points were obtained with a Thomas Hoover capillary melting point apparatus and were corrected. Ethyl ether was distilled from Na before use, THF from $LiAlH_4$, and methanol and *tert*-butyl alcohol from Mg. All apparatus was flame dried.

Methyl (*Z*)- and (*E*)-3-Carboxy-6-methyl-2-heptenoates (IIIb and IVb). To 13.2 g (1.90 g-atom) of lithium wire, flattened and cut into ~2-cm pieces, in 275 mL of ether under argon was added about 35 drops of a solution of 60.9 g (0.403 mol) of isoamyl bromide in 50 mL of ether. The reaction mixture was then cooled with a dry ice–acetone bath, the remainder of the isoamyl bromide was added over 45 min while maintaining a temperature between −35 and −40 °C, and then the mixture was allowed to warm to −10 °C while stirring for an additional 90 min. The reaction mixture was filtered under argon pressure through glass wool and the product was transferred into a precalibrated bottle, yielding 270 mL of 1.36 N isoamyllithium (0.367 mol), a 91% yield as determined by double titration.[12]

To a slurry of 9.52 g (0.050 mol) of cuprous iodide and 40 mL of ether under argon was added 104 mL of 0.97 N isoamyllithium (0.100 mol) over 15 min at −78 °C. Then 7.10 g (0.050 mol) of dimethyl acetylenedicarboxylate in 40 mL of ether was added to the cold stirring mixture over 15 min. After stirring for 3 h at −78 °C the reaction was quenched while still cold with methanol and neutralized with 3 N HCl. The organics were extracted with ether (centrifuging necessary), the ether layers were combined, dried over magnesium sulfate, and filtered, the ether was evaporated, and low-boiling organics were removed under reduced pressure. The crude product (10.5 g) by NMR contained 80% IIIb (NMR (CCl_4) 0.92 (6 H, d), 1.0–1.9 (3 H, m), 2.33 (2 H, t), 3.68 (3 H, s, MeOCOCR=), 3.75 (3 H, s, MeOCOCH=), 5.72 (1 H, t, J = 1.5 Hz)) and 10% IVb (NMR (CCl_4) 0.85 (6 H, d), 1.0–1.8 (3 H, m), 2.67 (2 H, ~t), 3.60 (3 H, s), 3.64 (3 H, s), 6.50 (1 H, s)). Reported[5] vinyl hydrogen shifts for IIIb and IVb prepared differently are δ 5.85 and 6.80, respectively. These isomers could be separated with 80% recovery by GC (0.25 in. × 7 ft Carbowax 20M on Chromosorb P at 200 °C) or by column chromatography (Silica, 4:3 cyclohexane–ethyl acetate); IVb moved faster in both cases.

(*Z*)- and (*E*)-3-Carboxy-6-methyl-2-heptenoic Acids (IIIc and IVc). To a 50-mL flask containing 30 mL of 3 N HCl was added 2.0 g of crude product from the dialkyl cuprate reaction (80% IIIb, 10% IVb). After refluxing for 8 h, the reaction mixture was cooled to 25 °C and filtered, yielding 0.121 g of diacid IVc, mp 204–205 °C, from $HCCl_3/CCl_4$ (lit.[5] mp 203–204 °C): NMR (Me_2CO-d_6) 0.93 (6 H, d), 1.0–1.9 (3 H, m), 2.78 (2 H, ~t), 6.71 (1 H, s), 10.6 (2 H, s). Following thorough ether extraction of the filtrate, the product was extracted with saturated aqueous sodium bicarbonate (unhydrolyzed ester remaining in the ether layer can be recovered and recycled). Acidification of the bicarbonate extracts with concentrated HCl was followed by extraction with ether. The ether layers were combined, dried over magnesium sulfate, and filtered, and the ether was evaporated, yielding 1.29 g of viscous oil that contained: 83% diacid IIIc, NMR (CCl_4) 0.92 (6 H, d), 1.0–1.9 (3 H, m), 2.40 (2 H, t), 5.80 (1 H, t, J = 1.2 Hz), 10.75 (2 H, s); 4% diacid IVc (vinyl H peak at δ 6.76); and 13% anhydride VI (vinyl H peak at δ 6.53 t, J = 1.7 Hz).

(*Z*)-3-Carboxy-6-methyl-2-heptenoic Anhydride (VI). Diacid IIIc was refluxed overnight with excess acetic anhydride. Distillation of excess acetic anhydride and acetic acid gave an essentially quantitative yield of anhydride VI: NMR (CCl_4) 0.95 (6 H, d), 1.2–1.9 (3 H, m), 2.50 (2 H, td, J = 7 Hz, 1.7 Hz), 6.53 (1 H, t, J = 1.7 Hz). Alternatively, diacid IVc was refluxed with a large excess of P_2O_5 in benzene for 2 h in a micro-Hickmann apparatus.[5] Evaporation of the benzene under a stream of argon followed by heating to 200 °C at 0.75 mm yielded virtually pure anhydride VI as a distillate.

Methyl (*Z*)-3-Carboxy-6-methyl-2-heptenoate (IIIa). When anhydride VI in CCl_4 was mixed with a sixfold excess of anhydrous methanol in an NMR tube complete reaction was indicated in 24 h to yield: 79% IIIa, NMR (CCl_4) 0.91 (6 H, d), 1.0–1.9 (3 H, m), 2.35 (2 H, t), 3.74 (3 H, s), 5.76 (1 H, t, J = 1.4 Hz), 11.60 (1 H, s); 13% IIId, NMR like IIIa except MeO at δ 3.67 and vinyl H at δ 5.79; and 8% Va (MeO at δ 3.65, CH_2 at δ 3.29, vinyl H at δ 7.07 t, J = 7.5 Hz). Similar results were obtained when anhydride VI was refluxed with a large excess of methanol for 3 h (77% IIIa, 19% IIId, and 4% Va).

3-Carbomethoxy-6-methyl-3-heptenoic Acid (Vd). To a refluxing solution of 4.30 g (0.110 g-atom) of potassium in 60 mL of anhydrous *tert*-butyl alcohol under argon was added over 15 min a mixture of 8.6 g (0.100 mol) of 3-methylbutanal and 19.5 g (0.133 mol) of dimethyl succinate. The reaction mixture was refluxed for an additional 1.5 h, then most of the solvent was removed under reduced pressure, the residue was made slightly acidic with 3 N HCl, the remaining solvent was removed, and the organics were ether extracted. The product was extracted into saturated aqueous sodium bicarbonate, the bicarbonate extracts were combined and made strongly acid with concentrated HCl, and the resulting mixture was extracted

with ether. Evaporation left 16.3 g (82%) of residual yellow oil which was very largely Vd: NMR (neat) 0.95 (6 H, d), ~1.8 (1 H, m), 2.07 (2 H, t), 3.38 (2 H, s), 3.68 (3 H, s), 6.97 (1 H, t, J = 7.5 Hz), 10.15 (1 H, s). This product was used without further purification.

3-Carboxy-6-methyl-3-heptenoic Acid (Vc). When 2.00 g of half-ester Vd from the previous reaction was refluxed overnight in 20 mL of 3 N HCl, white crystals formed. Recrystallization from chloroform yielded 1.47 g (79%) of diacid Vc: mp 164–165 °C; NMR (Me_2CO-d_6) 0.95 (6 H, d), ~1.8 (1 H, m), 2.17 (2 H, t), 3.36 (2 H, s), 7.00 (1 H, t, J = 7.5 Hz), 10.60 (2 H, s).

3-Carboxy-6-methyl-3-heptenoic Anhydride (VII). Diacid Vc was refluxed overnight with excess acetic anhydride. Distillation under reduced pressure to remove excess acetic anhydride and acetic acid gave an essentially quantitative yield of anhydride VII: NMR (neat) 0.97 (6 H, d), ~1.8 (1 H, m), 2.20 (2 H, ~t), 3.53 (2 H, ~s), 6.92 (tt, J = 7.5 and 2.6 Hz).

Methyl 3-Carboxy-6-methyl-3-heptenoate (Va). When a neat sample of anhydride VII was mixed with a twofold excess of anhydrous methanol in an NMR tube complete reaction was indicated in 24 h to yield half-ester Va which was by NMR 94% pure. Preparative TLC (silica: 55:45:2 ether–hexane–acetic acid) gave pure Va, NMR ($DCCl_3$) virtually identical to that reported.[4]

Equilibrium between Anhydrides VI and VII. Neat 0.5-g samples of VI and VII in separate NMR tubes with 1 drop of tri-n-butylamine were heated in increments of 25 °C from 25 to 150 °C for 10 min with ^{1}H NMR spectra being taken after each heating. By integration of the vinyl hydrogen signals at δ 6.5 (VI) and 6.8 (VII), the relative amounts of the two anhydrides were estimated. At 125 °C equilibrium was reached with 65% VII–35% VI in both tubes; at 150 °C, the equilibrium mixture contained 70% VII.

Methyl 3-Carbomethoxy-6-methyl-3-heptenoate (Vb). A. From Half-Ester Vd. After refluxing 2.00 g of Vd with 20 mL of methanol and 1 mL of acetyl chloride overnight, the neutral fraction from an extractive workup was distilled to give 1.77 g (83%) of diester Vb, bp 143 °C (22 mm): NMR (neat) 0.92 (6 H, d), ~1.8 (1 H, m), 2.12 (2 H, t), 3.33 (2 H, s), 3.57 (3 H, s), 3.65 (3 H, s), 6.93 (1 H, t, J = 7.5 Hz).

B. By Isomerization of IIIb and IVb. Refluxing 0.5 g of a mixture of 87% IIIb and 13% IVb with 10 mL of 1 M sodium methoxide in methanol overnight followed by an extractive workup and removal of solvent by distillation gave a quantitative yield of residual oil which had a ^{1}H NMR spectrum virtually identical with that of Vb prepared as described above.

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Registry No.—IIIa, 63731-47-5; IIIb, 51804-78-5; IIIc, 16110-97-7; IIId, 63731-48-6; IVb, 51804-76-3; IVc, 51804-75-2; Va, 63731-49-7; Vb, 63731-50-0; Vc, 63731-51-1; Vd, 63731-52-2; VI, 51804-77-4; VII, 63731-53-3; isoamyl bromide, 107-82-4; isoamyl lithium, 7488-31-5; dimethyl acetylenedicarboxylate, 762-42-5; 3-methylbutanal, 590-86-3; dimethyl succinate, 106-65-0.

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(11) We repeated the osmium tetroxide *syn*-hydroxylations of diesters IIIb and IVb,[5] obtaining the same products in higher yield (92% with IIIb and 72% with IVb after recrystallization, compared to 40 and 65%[5]) with pyridine as solvent.
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