90–95 °C; dec; $[\alpha]_D^{23}$ −16.4° (c 0.64, MeOH).

Anal. Calcd for $C_{29}H_{37}N_6O_{16}F_3$: C, 44.50; H, 4.77; N, 10.74. Found: C, 44.72; H, 4.93; N, 10.65.

N^{α}-Benzyloxycarbonyl-L-lysylglycyl-L-asparaginyl-L-leucyl-L-γ-carboxyglutamyl-L-arginyl-L-γ-carboxyglutamic Acid α-Methyl Ester p-Toluenesulfonate Trifluoroacetate Mixed Salt (17). A 0.025-g (0.016 mmol) sample of **15** was dissolved in 1 mL of a 50:50 mixture of glacial acetic acid and trifluoroacetic acid and allowed to stand at room temperature for 36 h. The solution was evaporated in vacuo and triturated with ethyl ether to yield 0.021 g (98%) of **17**: mp 63–65 °C.

Anal. Calcd for $C_{54}N_{77}O_{24}SF_3$: C, 47.43; H, 5.68; N, 12.29. Found: C, 47.82; H, 5.75; N, 12.03.

^{1}H NMR Studies on the Carboxylate–Guanidino Interaction of 16. A 0.95-g (0.025 mmol) sample of **16** was dissolved in 0.5 mL of Me_2SO-d_6 and the proton NMR spectrum recorded. Homonuclear decoupling experiments at the proton resonances corresponding to the δ-protons of arginine, the γ-protons of Gla, and the α-protons of all three residues were performed (Bruker Model WM250). One equivalent (0.0041 g, 0.012) mmol) of cesium carbonate was then added to the NMR tube and the solution placed under a vacuum to remove the carbon dioxide formed. The normal proton spectrum and the corresponding homonuclear decoupled spectra were recorded. These same spectra were also recorded at concentrations of **16** of 25 mM, 10 mM, 5 mM, and 1 mM.

Acknowledgment. This research was supported by Grant HL-20161 from the National Heart, Lung, Blood Institute, U.S. Public Health Service. We also acknowledge the contributions of Dr. David L. Harris and Ms. M. W. Pendergraft.

Registry No. 1, 60686-50-2; 2, 64153-37-3; 3, 87136-42-3; 4, 76804-70-1; 5, 76822-52-1; 6, 87173-10-2; 7·H_3CCO_2H, 87136-44-5; 8, 10342-52-6; 9, 14317-83-0; 10, 47689-13-4; 11, 29359-35-1; 12, 87136-45-6; 13·Li, 87136-46-7; 14, 87136-47-8; 15·Me-p-$C_6H_4SO_3H$, 87136-49-0; 16·F_3CCO_2H, 87136-51-4; 17·F_3CCO_2H·Me-p-$C_6H_4SO_3H$, 87136-53-6; H-Arg-OH, 74-79-3; Z-Lys(BOC)-OH·DCHA, 2212-76-2; H-Gly-OMe·HCl, 5680-79-5; N-(benzyloxycarbonyl)-L-aspargine, 2304-96-3; L-leucine methyl ester hydrochloride, 7517-19-3; vitamin K, 12001-79-5.

Novel Biogenetic Pathways from (+)-Reticuline. Three Dimeric Alkaloids: (+)-Vanuatine, (+)-Vateamine, and (+)-Malekulatine

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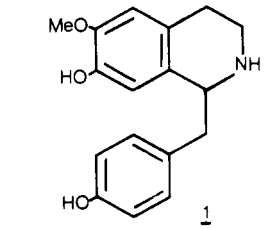
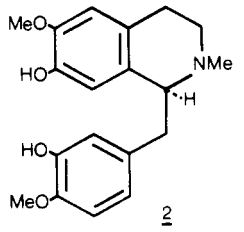
The bark of *Hernandia peltata* Meissner (Hernandiaceae), gathered in the Republic of Vanuatu (New Hebrides), has yielded the bis(benzylisoquinolines) (+)-vanuatine (**5**), (+)-vateamine (**6**), and (+)-malekulatine (**7**). These are the first-known dimers of (+)-reticuline (**2**). Compounds **5** and **6** are products of tail-to-tail oxidative coupling, whereas **7** involves head-to-tail coupling.

The isoquinoline skeleton is incorporated into well over 1000 alkaloids, and most of these compounds may be broken down into two broad categories depending upon whether they are derived biogenetically from the tetrahydrobenzylisoquinolines (±)-coclaurine (**1**) or (+)-reticuline (**2**). (±)-Coclaurine-derived alkaloids tend to dimerize to provide more than 250 different bis(benzylisoquinolines).[1] Alternatively, they may undero intramolecular oxidative coupling to proaporphines, which can rearrange to aporphines.



1



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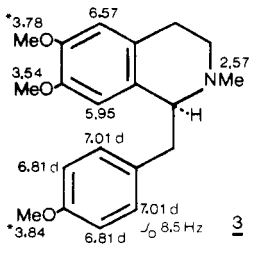

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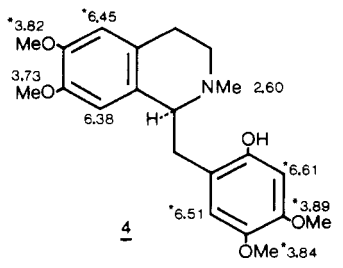


4

The in vivo chemistry of (+)-reticuline (**2**), which possesses an additional oxygen in the bottom ring, differs in some important respects from that of (±)-coclaurine (**1**). There is a proclivity for internal cyclization, due to the added activation of the bottom ring, to generate a pavine, an isopavine, a dibenzopyrrocoline, a berbine, or an aporphine. The tendency to dimerize is minimal, and in fact only nine bis(benzylisoquinolines) are known that incorporate a reticuline moiety bonded to a coclaurine unit, while none were known that originate from bonding between two reticulines.[1,2]

We now report the first three bis(benzylisoquinoline) alkaloids derived from the condensation of two (+)-reticuline units. Of added significance is the fact that each of these three dimers is formed by a different mode of

(1) Guha, K. P.; Mukherjee, B.; Mukherjee, R. *J. Natural Products* **1979**, *42*, 1. Schiff, P. L., Jr. *Ibid.* **1983**, *46*, 1.

(2) Some 30 aporphine–benzylisoquinoline alkaloids of the thalicarpine type are known. These alkaloids are formed biogenetically through coupling of a fully formed aporphine unit with (+)-reticuline (**2**). See: Sidjimov, A. K.; Marekov, N. L. *Phytochemistry* **1982**, *21*, 871. Bhakuni, D. S.; Jain, S. *Tetrahedron* **1982**, *38*, 729.

0022-3263/83/1948-3957$01.50/0

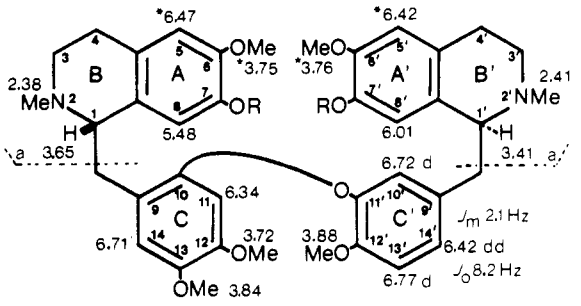
Table I. 360-MHz (FT) NMR Chemical Shifts (δ) in $CDCl_3$ for the O-Methylated Alkaloids

	methyl		methoxyl		aromatic proton									
	*N-2	*N-2′	+C-7	+C-7′	**C-5	**C-5′	*C-8	*C-8′	C-10′[a]	C-11	C-13	C-13′[b]	C-14	C-14′[c]
8	2.43	2.44	3.55	3.61	6.48	6.54	6.13	6.14	6.46	6.48		6.87	6.58	6.76
9	2.39	2.38	3.55	3.56	6.45	6.53	6.05	6.06	6.40		6.70	6.82	6.77	6.59
10	2.46	2.49	3.50		6.44	6.48	6.04	6.23	6.56	6.66		6.64	6.56	6.52

[a] d, J_m = 2.44 Hz. [b] d, J_o = 8.24 Hz. [c] dd, J_m = 1.83 Hz and J_o = 8.24 Hz. [d] d, J_o = 8.54 Hz. Other methoxyl singlets for 8: δ 3.73, 3.77, 3.79, 3.82, and 3.86; for 9, δ 3.71, 3.78, 3.82, 3.86 and 3.90; for 10, δ 3.72, 3.74 (6 H), 3.81, 3.83, and 3.84. Chemical shifts with identical superscripts are interchangeable.

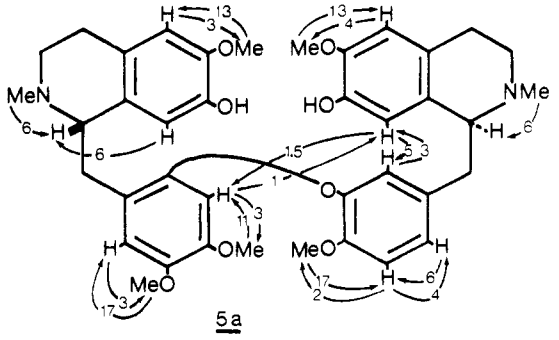
phenolic oxidative coupling. Vanuatine (**5**) and vateamine (**6**) evince tail-to-tail coupling para to an original phenolic function in one case, and ortho in the other. In contrast, malekulatine (**7**) is formed through head-to-tail coupling.

Extraction and chromatography of the methanol extracts from 2.4 kg of ground bark of *Hernandia peltata* Meissner (Hernandiaceae), collected in the Republic of Vanuatu (New Hebrides) yielded 1.0 g of the diphenolic (+)-vanuatine (**5**), $C_{39}H_{46}O_8N_2$, whose UV spectrum with a maximum at 286 nm is close to that of several tetrahydroisoquinolines.[1] The mass spectrum, with a small molecular ion at m/z 670 and a dominating base peak at m/z 192 was immediately suggestive of a dimer involving tail-to-tail coupling. Facile benzylic cleavage then generates base ion a due to rings A and B, as well as to A′ and B′.



5 R=H ; 8 R=Me (nmr is for 5)

The NMR spectrum of vanuatine incorporates five methoxyl singlets and has been summarized around expression **5**. Particularly significant are the relatively upfield aromatic singlets at δ 5.48 and 6.01 assigned to H-8 and H-8′, respectively, and reminiscent of the chemical shifts for H-8 in a monomeric N-methylated tetrahydrobenzylisoquinoline. The chemical shift assignments were confirmed by an NMR NOEDS (nuclear Overhauser enhancement difference study)[3] whose results have been outlined around expression **5a**. Of interest is the finding

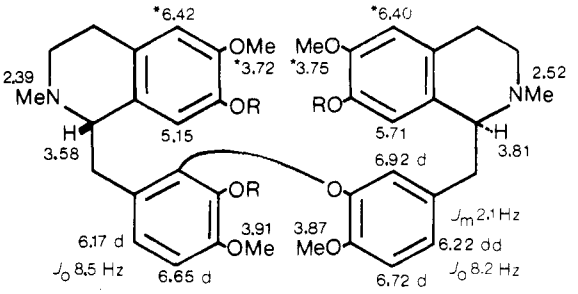


5a

that irradiation of H-8′ (δ 6. 01) results in a 5% NOE of H-10′ (δ 6.72) and a 1.5% NOE of H-11 (δ 6.34) so that ring A′ is located close to rings C′ and C.

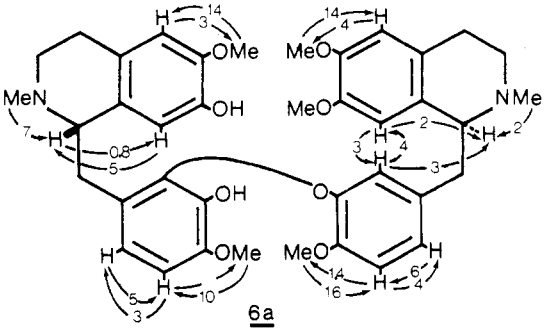
Diazomethane O-methylation of vanuatine yielded (+)-*O*,*O*-dimethylvanuatine (**8**), $C_{41}H_{50}O_8N_2$, whose NMR spectrum contains two additional three-proton singlets upfield at δ 3.53 and 3.61, diagnostic of the C-7 methoxyl of an N-methylated tetrahydrobenzylisoquinoline (Table I).[4] Finally, sodium in liquid ammonia cleavage of *O*,*O*-dimethylvanuatine (**8**) provided (+)-*O*-methylarmepavine (**3**) and base **4**, whose negative specific rotation indicates that ring C is turned toward the *N*-methyl group. Both **3** and **4** have CD spectra with a positive maximum near 233 nm, pointing to the *S* configuration.[5]

The triphenolic (+)-vateamine (**6**), $C_{38}H_{44}O_8N_2$, 0.52 g, has a UV spectrum with a maximum at 283 nm. The mass spectrum, besides showing a small molecular ion at m/z 656, resembles that of vanuatine in that again there is a paramount base peak at m/z 192.



6 R=H ; 9 R=Me (nmr is for 6)

The NMR spectrum of (+)-vateamine, beside indicating the presence of only four methoxyls, differs from that of vanuatine mainly in the aromatic region. The two protons of ring C are represented by a doublet of doublets at δ 6.17 and 6.65 (J_o = 8.5 Hz) rather than by two singlets as in the spectrum of vanuatine. The assignments of chemical shifts was further ascertained by an NMR NOEDS whose results are presented around expression **6a**.



6a

O-Methylation of vateamine produced (+)-*O*,*O*,*O*-trimethylvateamine (**9**), $C_{41}H_{50}O_8N_2$, whose NMR spectrum contains seven methoxyl singlets, two of which are relatively upfield (δ 3.55 and 3.56) since they represent the C-7

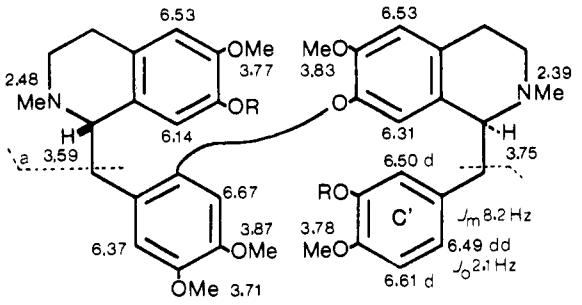
(3) Hall, L. D.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1980**, *102*, 5703.

(4) A conformational change must take place upon *O*-methylation of vanuatine (**5**) and vateamine (**6**). The NMR spectral absorption for H-8, which is appreciably upfield for these two alkaloids (δ 5.00–5.50) appears downfield in the *O*-methylated derivatives **8** and **9** (δ 6.05–6.15).

(5) Craig, J. C.; Martin-Smith, M.; Roy, S. K.; Stenlake, J. B. *Tetrahedron* **1966**, *22*, 1335.

and C-7′ substituents (Table I).[4] Sodium in liquid ammonia reduction of **9** proceeded cleanly to supply (+)-laudanidine (**11**) and (+)-laudanosine (**12**).

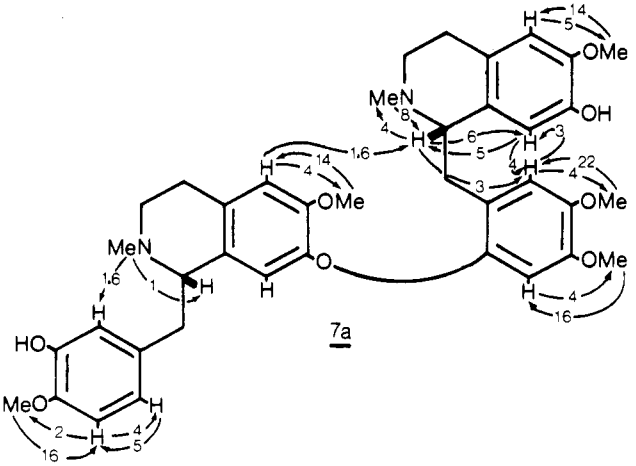
It was immediately apparent, from simple inspection of the mass spectrum, that the third alkaloid isolated, namely, the diphenolic (+)-malekulatine (**7**), $C_{39}H_{46}O_8N_2$, 2.3 g, is



7 R=H ; **10** R=Me (nmr is for **7**)

radically different in its bonding from the other two dimers. There is a weak molecular ion at m/z 670. The base peak is still at m/z 192 but is not as dominant as in the two instances discussed above since there is also an important peak at m/z 533 (8%) which corresponds to loss of ring C′ from the molecular ion. The implication is thus that head-to-tail rather than tail-to-tail coupling is involved.

As with the previous two alkaloids, the NMR spectral assignments were confirmed by NOE measurements as summarized in **7a**. Only upon *O*-methylation to supply



7a

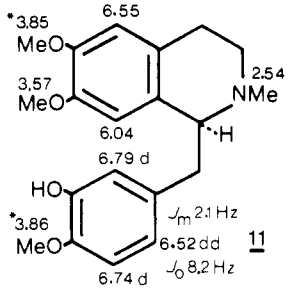
(+)-*O*,*O*-dimethylmalekulatine (**10**), $C_{41}H_{50}O_8N_2$, did the NMR spectrum show an upfield *O*-methyl singlet at δ 3.50, diagnostic of a C-7 methoxyl (Table I).

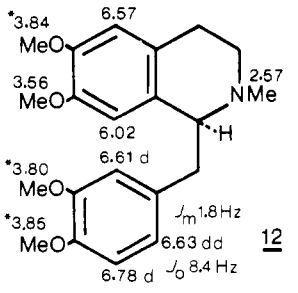
Conclusive proof of structure was again provided by a sodium in liquid ammonia reduction of **10**, which led to the tetrahydrobenzylisoquinoline (+)-**13**, as well as to the phenolic tetrahydrobenzylisoquinoline (−)-**4**, previously obtained from the reduction of *O*,*O*-dimethylvanuatine (**8**).

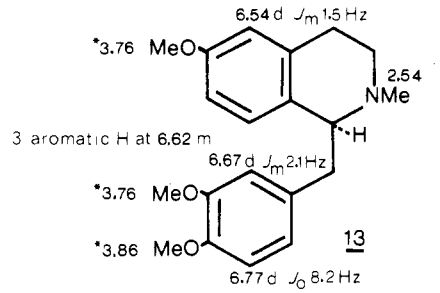
A rule governing the dimerization of benzylisoquinolines in nature is that initial coupling occurs either in tail-to-tail or in head-to-tail fashion and never head to head. This rule must apply also to the dimers of (+)-reticuline (**2**), as exemplified by the alkaloids vanuatine (**5**), vateamine (**6**), and malekulatine (**7**).

Experimental Section

Standard Experimental Conditions. NMR spectra are at 360 MHz (FT) in $CDCl_3$ solution. The NOE experiments were carried out by FT NOE difference spectroscopy that allows enhancements as low as 0.5% to be observed. The equilibration time was at least ten times T_1. The samples were degassed prior







to the measurements.[6] Molecular compositions were confirmed by high-resolution mass spectroscopy.

Alkaloid Extraction and Purification. *Hernandia peltata* Meissner was collected in the Republic of Vanuatu on July 24, 1980. A sample has been preserved at the ORSTOM, with number PCNH-1079.

The powdered bark (2.4 kg) was first extracted with petroleum ether and then quickly washed with chloroform. After drying, the powder was extracted with methanol by using a Soxhlet extractor. The methanolic solution was concentrated and stirred manually with 1% hydrochloric acid. The aqueous solution was basified and reextracted with chloroform to furnish 40.8 g of total alkaloids.

An initial separation was achieved on a Sephadex LH-20 column using chloroform–methanol (30:70) as eluent. Four fractions were collected. The first was nonalkaloidal (1.5 g), the second was rich in dimers (12.4 g), and the third consisted of monomeric alkaloids and nonalkaloids (2.4 g).

The second fraction, which was rich in dimers, was subjected to column chromatography using Sephadex LH-20 as above to supply 9.5 g of fairly pure dimeric alkaloids. These alkaloids were dissolved in chloroform–ether, and the solution was extracted with dilute aqueous sodium hydroxide to separate the phenolic compounds (7 g) from the nonphenolic bases (0.8 g).

The phenolic alkaloids were chromatographed on a column containing silica gel for TLC, using the solvent chloroform–methanol (1.75:0.25, v/v). The following fractions were collected: impure vanuatine (**5**), 0.27 g; pure vanuatine (**5**), 0.43 g; vanuatine (**5**) and malekulatine (**7**), 0.68 g; malekulatine (**7**), 1.33 g; malekulatine (**7**) and (+)-reticuline (**2**), 1.87 g; vateamine (**6**), 0.52 g.

The mixture consisting of 0.68 g of vanuatine and malekulatine was fractionated on a column of silica gel for TLC, using benzene–acetone–ammonium hydroxide (30:60:1). Final purification of each alkaloid was by preparative TLC.

General Conditions for *O*-Methylation. A sample of the alkaloid ($\simeq$2 mg) was dissolved in 2 mL of methanol. To this solution was added excess diazomethane in ether–methanol. The yellow solution was allowed to stand in the refrigerator for 24 h. Workup involved purification by TLC on silica gel.

General Conditions for Sodium in Liquid Ammonia Reduction. In a 100-mL, three-necked flask, $\simeq$10 mg of the alkaloid was dissolved in 10 mL of THF, and 10 mL of liquid ammonia was added at about −78 °C. The reaction vessel was equipped with a Dewar-type condenser, and the system was kept under nitrogen. A minimum amount of sodium was added as small chunks, with stirring, so as to produce a stable blue color lasting for at least 0.5 h but for less than 2 h. The reaction mixture was

(6) In diagrams **3**–**6** and **11**–**13**, chemical shift values with identical superscripts are interchangeable.

allowed to warm up to room temperature, and excess methanol was added to destroy any residual sodium. Following removal of the solvent, the residue was treated with water and acidified with dilute hydrochloric acid. The mixture was then basified with ammonium hydroxide and extracted with chloroform. The organic phase was dried over sodium sulfate, the solvent evaporated, and the residue subjected to preparative TLC. Average yield: 70% of each component.

(+)-*O*-Methylarmepavine (3): $[\alpha]^{25}_D$ +87° (*c* 0.15, MeOH); CD $\Delta\epsilon_{nm}$ (MeOH) $+1.2_{287}$, $+5.0_{234}$.

Phenolic Tetrahydrobenzylisoquinoline 4: MS, *m/z* (relative intensity) 373 (M^+, $C_{21}H_{27}O_5N$, 0.4), 356 (0.7), 206 (100), 191 (6), 190 (15), 177 (3); λ_{max} (MeOH) 210, 231 sh, 288 nm (log ϵ 4.45, 4.15, 3.89); CD $\Delta\epsilon_{nm}$ (MeOH) -2.9_{295}, $+2.0_{279}$, -4.3_{243}, $+3.9_{232}$; $[\alpha]^{25}_D$ −132° (*c* 0.22, MeOH).

(+)-Vanuatine (5): MS, *m/z* (relative intensity) 670 (M^+, 0.2), 669 (1), 655 (0.4), 535 (0.1), 478 (0.8), 477 (1), 341 (0.1), 192 (a, 100); λ_{max} (MeOH) 210, 230 sh, 286 nm (log ϵ 4.83, 4.48, 4.11); CD $\Delta\epsilon_{nm}$ (MeOH) $+6.2_{289}$, $+23.2_{232}$; $[\alpha]^{25}_D$ +138° (*c* 0.12, MeOH).

(+)-Vateamine (6): MS, *m/z* (relative intensity) 656 (M^+, 0.1), 655 (0.2), 519 (0.1), 464 (0.3), 327 (0.2), 192 (100); λ_{max} (MeOH) 212, 230 sh, 283 nm (log ϵ 4.72, 4.48, 4.07); CD $\Delta\epsilon_{nm}$ (MeOH) $+7.5_{286}$, $+14.6_{233}$; $[\alpha]^{25}_D$ +204° (*c* 0.14, MeOH).

(+)-Malekulatine (7): MS, *m/z* (relative intensity) 670 (M^+, 0.1), 669 (0.2), 533 (8.4), 478 (0.1), 192 (100); λ_{max} (MeOH) 211, 230 sh, 284 nm (log ϵ 4.79, 4.50, 4.18); CD $\Delta\epsilon_{nm}$ (MeOH) $+5.8_{282}$, $+26.5_{232}$; $[\alpha]^{25}_D$ +156° (*c* 0.14, MeOH).

(+)-*O,O*-Dimethylvanuatine (8): MS, *m/z* (relative intensity) 698 (M^+, 0.2), 492 (0.5), 206 (100); CD $\Delta\epsilon_{nm}$ $+5.2_{287}$, $+28.4_{235}$; $[\alpha]^{25}_D$ +78° (*c* 0.12, MeOH).

(+)-*O,O,O*-Trimethylvateamine (9): MS, *m/z* (relative intensity) 698 (M^+, 0.1), 492 (0.4), 206 (100); CD $\Delta\epsilon_{nm}$ $+3.2_{284}$, $+11.3_{235}$; $[\alpha]^{25}_D$ +118° (*c* 0.2, MeOH).

(+)-*O,O*-Dimethylmalekulatine (10): MS, *m/z* (relative intensity) 698 (M^+, 0.4), 547 (38), 492 (0.2), 206 (100); CD $\Delta\epsilon_{nm}$ $+3.05_{283}$, $+14.9_{236}$.

(+)-Laudanidine (11): CD $\Delta\epsilon_{nm}$ (MeOH) $+4.5_{288}$, $+9.3_{238}$; $[\alpha]^{25}_D$ +72° (*c* 0.3, MeOH).

(+)-Laudanosine (12): CD $\Delta\epsilon_{nm}$ (MeOH) $+1.2_{286}$, $+3.5_{237}$; $[\alpha]^{25}_D$ +80° (*c* 0.1, MeOH).

Trimethoxytetrahydrobenzylisoquinoline 13: MS, *m/z* (relative intensity) 327 (M^+, $C_{20}H_{25}O_3N$, 0.2), 310 (0.4), 190 (2), 176 (100); CD $\Delta\epsilon_{nm}$ (MeOH) $+0.8_{280}$ $+4.4_{230}$; $[\alpha]^{25}_D$ +70° (*c* 0.1, MeOH).

Acknowledgment. M.S. is the recipient of Grant CHE-8210699 from the National Science Foundation.

Registry No. **2**, 485-19-8; **3**, 3423-02-7; **4**, 4829-27-0; **5**, 87183-74-2; **6**, 87183-75-3; **7**, 87183-76-4; **8**, 87183-77-5; **9**, 87183-78-6; **10**, 87183-79-7; **11**, 3122-95-0; **12**, 2688-77-9; **13**, 87246-83-1.

Enantioselective Hydrolysis of 3-Hydroxy-3-methylalkanoic Acid Esters with Pig Liver Esterase[1]

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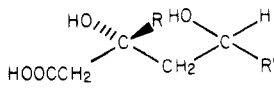
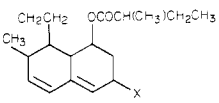
Pig liver esterase has been shown to stereoselectively hydrolyze the *R* enantiomer of several chiral 3-hydroxy-3-methylalkanoic acid esters of the form $RC(Me)(OH)CH_2COOR'$, where R = Et, $CH_2{=}CHCH_2$, $Me(CH_2)_5$, $(MeO)_2CHCH_2$, and $PhCH_2OCH_2CH_2$ and R′ = Me or Et. The unhydrolyzed ester and the reesterified carboxylic acid were analyzed for enantiomeric purity by NMR using the chiral shift reagent $Eu(hfc)_3$. For the compounds studied, the *S* enantiomers consistently showed greater induced shifts. Products of the resolution are potential intermediates in the preparation of compactin analogues having defined stereochemistry at carbon-3. These analogues will be useful in testing the hypothesis that the hypocholesterolemic activity of compactin and its analogues resides in their ability to mimic the binding of mevaldic acid coenzyme A hemithioacetal to HMG-CoA reductase but not be reduced to mevalonate.

During the past several years, a number of 3,5-dihydroxyalkanoic acids have been reported to strongly inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the key regulated enzyme in sterol biosynthesis. Most notable among these are compactin **1a**)[3,4] and mevinolin (**1b**).[5,6] However, analogues, **2**, of these that have substituted aromatic rings in place of the hexahydronaphthalene of compactin and mevinolin have also proven to be potent HMG-CoA reductase inhibitors.[7] We feel that the ability of 3,5-dihydroxyalkanoic acids to inhibit HMG-CoA reductase resides in their ability to mimic the binding characteristics of either mevaldic acid coenzyme A hemithioacetal, (**3**, (the proposed[8] intermediate in the two-step reduction of HMG-CoA to mevalonic





1, R = H; R′ =
a, X = H;
b, X = CH_3
2a, R = H; R′ = CH_2CH_2Ar;
b, R = CH_3; R′ = CH_2CH_2Ar
3, R = CH_3; R′ = S-CoA

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