Journal of Medicinal Chemistry

Volume 28, Number 6 June 1985

Synthesis of Pyrrolizidine Alkaloids Indicine, Intermedine, Lycopsamine, and Analogues and Their *N*-Oxides. Potential Antitumor Agents

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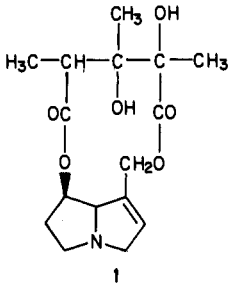
(−)- and (+)-trachelanthic and (−)- and (+)-viridifloric acids were synthesized and their isopropylidene derivatives were regiospecifically coupled, at C-9, with (−)-retronecine (2) obtained by hydrolysis of monocrotaline (1), isolated from *Crotalaria spectabilis*. Hydrolysis, followed by oxidation, led to the *N*-oxides of indicine (7), intermedine (13), lycopsamine (15), and the new nonnatural product 16, respectively. Each of these analogues was screened in the P388 lymphocytic leukemia system at the same time as indicine *N*-oxide, and the results were compared. Other related analogues were prepared and similarly screened and the results compared with those from indicine *N*-oxide.

The antitumor activity of the pyrrolizidine alkaloids has been recognized for about 20 years.[1,2] Culvenor[3] first observed that the active compounds were not significantly cytotoxic in cell culture and the in vivo activity was particularly noteworthy in the Walker 256 system, which is known to be sensitive to alkylating agents. These workers concluded that the same functionalities, in particular, an allylic oxygen function, were responsible for both hepatotoxicity and antitumor activity. Schoental[4] first reported that hepatotoxicity was related to unsaturation (see 1, monocrotaline) and soon thereafter, Culvenor et al.[5] proposed that alkylation of biological nucleophiles in the liver was responsible for the toxicity, after showing that C-1 allylic esters could be displaced by nucleophiles. In 1968, Mattocks[6] demonstrated that "metabolic pyrroles" produced in the liver were more reactive than the parent alkaloids to alkylation and there was a good correlation between hepatotoxicity and the amount of "metabolic pyrrole" produced.[7] Dehydroretronecine (9) has been shown to produce the same pattern of lesions in vivo as its macrocyclic diester parent, monocrotaline (1),[8] and esters of heliotridine (4) are metabolically converted into dehydroheliotridine (3) in vivo.[9] The "metabolic pyrroles" have been postulated as arising by C-hydroxylation at the C-3 allylic position in the pyrrolizidine nucleus, followed by elimination of water to give the dehydroalkaloid.[7] This metabolic system, however, is known not to be the same as the one that oxidizes the free bases to *N*-oxides.[10] Dehydroretronecine (9) has been shown to produce covalent adducts at C-7 with the thiol groups of cysteine and glutathione.[11] [^{3}H]Dehydroretronecine shows significant binding to bovine serum albumin and to calf thymus DNA in vitro with greatly increased binding at lower pH.[12] In vivo experiments have shown that binding to protein is much greater than to nucleic acids.[12,13]



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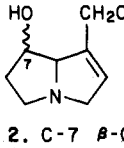
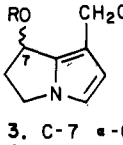


2. C-7 β-OH
4. C-7 α-OH



3. C-7 α-OH
9. C-7 β-OH

It has been suggested that pyrrolizidine *N*-oxides per se are not hepatotoxic[14] and their toxicity might arise only to the extent that they are converted to their corresponding bases. The route of drug administration would then be crucial, since reduction of *N*-oxides to free bases takes place in the gastrointestinal tract after oral administration.[15] A comparison of the toxicity of heliotrine (5), with its *N*-oxide (6), by intraperitoneal (ip) administration to the rat, showed acute LD_{50} of 300 mg/kg for the former and 5000 mg/kg for the latter, indicating that this *N*-oxide was only minimally converted to its free base.[16]

Indicine (7) and its *N*-oxide (8) were first isolated from

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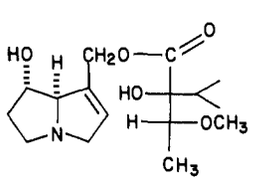
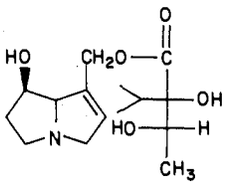


5
6, N-oxide



7
8, N-oxide

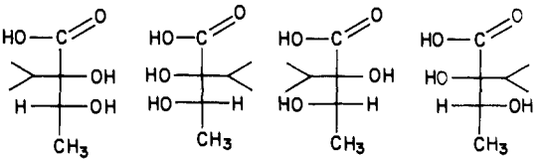
Heliotropium indicum in 1961 as part of an investigation of potentially hepatotoxic plants.[17] In 1976, the antitumor activity of indicine *N*-oxide (**8**) was discovered by a bioassay-directed fractionation of *H. indicum*.[18] Indicine *N*-oxide (**8**), given ip is a more active antitumor agent than indicine (**7**) or heliotrine *N*-oxide (**6**) and indicine *N*-oxide administered orally is inactive.[19] Thus, indicine is not responsible for the antitumor activity of indicine *N*-oxide. A comparison of the extent of metabolism and urinary excretion of indicine *N*-oxide (**8**) and heliotrine *N*-oxide (**6**) reveals the importance of subtle structural changes. Thus, 24 h after ip administration of indicine *N*-oxide, 100% could be accounted for in the urine as unchanged *N*-oxide (97%), indicine (2%), and indicine conjugates (1%). Under identical conditions, only 45% of heliotrine *N*-oxide could be accounted for, with 9.7% of this as heliotrine *N*-oxide conjugates. After oral administration, recovery of indicine *N*-oxide and metabolites was only 77.1% of which 26.9% was unchanged *N*-oxide, 0.6% was *N*-oxide conjugates, 37.4% was free indicine, and 12.2% was conjugated indicine.[19]

Indicine *N*-oxide has progressed to clinical studies at the National Cancer Institute, and these studies are continuing. The activity of indicine *N*-oxide in leukemia is significant, and the responses seen have been in patients who have failed induction of remission with the best standard agents, generally in combination. The two major toxicities seen to date were severe unpredictable myelosuppression and hepatotoxicity. Indicine *N*-oxide is a drug with good patient acceptance, since it does not cause nausea, vomiting, fever, rashes, or other discomforts seen with many anticancer agents.[15] The mechanism of the antitumor activity of indicine *N*-oxide is unclear at this time. It has been suggested that while the reduction of indicine *N*-oxide occurs only to a small extent after ip administration to mice[19] or rabbits[20] or intravenous (iv) administration in monkeys[21] or the human,[20] there could be increased reduction of indicine *N*-oxide in hypoxic tumor cells which would also be more acidic, leading to site specificity of production of metabolic pyrroles and selectivity to tumor cells over normal cells.[15]

In view of the antitumor activity of indicine *N*-oxide, its toxicities as observed in the clinic, and the differences in the metabolism and urinary excretion between the related *N*-oxides of indicine and heliotrine, we undertook this investigation to prepare all of the necic acid isomers of indicine *N*-oxide and some related analogues in sufficient quantities for in vivo screening in the P388 lymphocytic leukemia system in direct side by side comparison with indicine *N*-oxide.

Chemistry. Our synthetic procedure evolved around the coupling of optically pure synthetic necic acids with the optically pure necine retronecine (**2**). Because of the quantity needed, we felt that none of the elegant total syntheses of retronecine in the literature were suitable for our needs.[22-27] With some modification of the procedure previously described,[28] we are now able to obtain relatively large amounts of retronecine (**2**) from readily available natural monocrotaline (**1**) very quickly. Next, we turned our attention to the preparation of the optically pure necic acids. (±)-Trachelanthic acid was prepared by hydroxylation of *trans*-α-isopropylcrotonic acid[29,30] with osmium tetraoxide in the presence of chloric acid according to the procedure of Kochetkov et al.[31] (±)-Viridifloric acid was prepared as previously described[31,32] by the hydroxylation of *trans*-α-isopropylcrotonic acid with tungsten trioxide and 30% hydrogen peroxide. Previous workers have reported the resolution of (±)-trachelanthic and (±)-viridifloric acid with use of brucine[30,32] and α-phenylethylamine.[31] In our hands the use of (+)-α-phenylethylamine and (−)-α-phenylethylamine gave better results. Thus, the recrystallized salt from (+)-α-phenylethylamine and (±)-viridifloric acid gave, on acid hydrolysis, (+)-(2*R*,3*R*)-viridifloric acid (**10**), while the salt from (−)-α-phenylethylamine gave (−)-(2*S*,3*S*)-viridifloric acid (**11**). Kochetkov et al.[31] mistakenly report the reverse of these results in their paper. In a similar manner, (+)-α-phenylethylamine with (±)-trachelanthic acid deposited a salt which, after recrystallization and hydrolysis, yielded (−)-(2*R*,3*S*)-trachelanthic acid (**12**), while the use of (−)-α-phenylethylamine provided (+)-(2*S*,3*R*)-trachelanthic acid (**13**). After completion of this phase of our work, new stereoselective syntheses of viridifloric and trachelanthic acids were reported,[33,34] but these do not appear to offer any immediate practical advantages over the procedures utilized.

Having in hand the requisite necine, retronecine (**2**), and necic acids **10–13**, our next goal was the regiospecific coupling of the two components at C-9 of retronecine. In addition to indicine (**7**), two of the three remaining isomers are known natural products. Intermedine (**14**) is the C-9



10 11 12 13

14, C-9 ester of 13 with 2
15, C-9 ester of 11 with 2
16, C-9 ester of 10 with 2

ester of retronecine and (+)-trachelanthic acid (**13**), while

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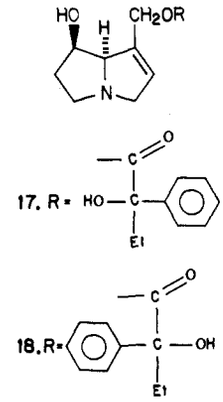
lycopsamine (**15**) is the C-9 ester of retronecine and (−)-viridifloric acid (**11**). The C-9 ester of retronecine and (+)-viridifloric acid (**16**) has never been reported either as a natural product or synthetically. Culvenor and Smith[35] reconstituted intermedine (**14**) and lycopsamine (**15**) by treating 1-(chloromethyl)-1,2-dehydro-7β-hydroxy-8α-pyrrolizidine, prepared by treating retronecine (**2**) with thionyl chloride,[36] with the sodium salts of trachelanthic and viridifloric acids recovered from hydrolysis of the alkaloids intermedine and lycopsamine, respectively. This work is of historical significance since it was the first reported synthesis of hepatoxocic pyrrolizidine alkaloids. However, no yields were given and a recent attempt to utilize this procedure gave unsatisfactory results.[37] Recently, Piper et al.[37] reported the synthesis of ^{3}H-labeled indicine *N*-oxide by the coupling of the isopropylidene derivative of (−)-trachelanthic acid, obtained by hydrolysis of indicine, with retronecine, labeled in the hydroxymethyl group, and also derived from indicine, using *N,N'*-dicyclohexylcarbodiimide (DCC) in the presence of 4-(dimethylamino)pyridine (DMAP) in toluene, followed by hydrolysis of the acetonide. A 50% yield of indicine, as a viscous colorless oil, was reported after TLC purification. Indicine, intermediate, and lycopsamine, as true for many pyrrolizidine alkaloids, are notorious for their propensity not to crystallize. Thus, intermedine was first reported almost 20 years ago and has been isolated from a number of sources[38,39] but only recently was it reported crystalline.[40] In 1983, X-ray crystal structure determinations were finally reported for intermedine and lycopsamine.[41]

Recently, there has been intensified interest in intermedine and lycopsamine as human health hazards in herbal teas[42] and in honey.[43] Since intermedine and lycopsamine commonly cooccur in plants, interest in their separations has recently taken advantage of high-performance liquid chromatography,[44,45] ion-pair absorption chromatography,[46] and chromatography of their borate complexes.[47] Recent advances in mass spectrometry[48] and ^{13}C NMR[49,50] have been used in their analyses, and as discussed below, the use of 300-MHz ^{1}H NMR spectroscopy permits one to distinguish between indicine (**7**) and all of its isomers (**14–16**).

Attempts to couple the isopropylidene derivatives of the necic acids **10–13** with retronecine according to the procedure of Piper et al.,[37] as previously mentioned, failed, in our hands, to give decent yields with use of toluene, chloroform, or ether as solvent integration in the absence or presence of DMAP. In the absence of DMAP, no coupling at all was observed. Better results were obtained with *N,N'*-carbonyldiimidazole (CDI) as the coupling agent according to the procedure of Hoskins and Crout[51] but substituting DMF for THF as the solvent. We recently reported the use of CDI in the synthesis of the semisynthetic analogues 9-*O*-[(*S*)-(+)-2-hydroxy-2-phenylbutyryl)]retronecine (**17**) and 9-*O*-[(*R*)-(−)-2-hydroxy-2-phenylbutyryl]retronecine (**18**).[28] Thus, the isopropylidene derivatives of (+)-(2*S*,3*R*)-viridifloric acid,[53] (−)-(2*S*,3*S*)-viridifloric acid,[52] (−)-(2*R*,3*S*)-trachelanthic acid, and (+)-(2*S*,3*R*)-trachelanthic acid,[55,56] respectively, were prepared as previously described by Piper et al.[37] for (−)-trachelanthic acid, and then they were coupled with retronecine with use of CDI and imidazoylsodium in a small volume of DMF. Interestingly, no coupling was observed in chloroform, THF, or ether and only a few percent reaction was observed in DMF, Me_2SO, or HMPA in the absence of imidazoylsodium. In the presence of the latter in DMF, yields of 50–70% were obtained, and the reaction was regiospecific, giving no detectable amounts of the C-7 esters or diesters.



As might be expected, when the coupling reaction was carried out with retronecine (**2**) in the presence of 3 equiv of the isopropylidene derivative of racemic trachelanthic acid, unequal amounts of the two diastereomeric coupled products were obtained as determined by integration of the C-9 proton signals of the deprotected esters in the NMR (see below for discussion of NMR data).

The unprotected esters indicine (**7**), intermedine (**14**), lycopsamine (**15**), and **16** were obtained by hydrolysis of the protected esters with 0.6 N HCl followed by basification and extraction with chloroform. A short chromatography gave analytically pure material. The present syntheses of the four isomers indicine (**7**), intermedine (**14**), lycopsamine (**15**), and the new isomer **16** and their corresponding isopropylidene derivatives by the procedure outlined permitted us to investigate the use of 300-MHz high-resolution ^{1}H NMR spectroscopy in $CDCl_3$ as a solvent, as a tool to distinguish between these diastereomers.

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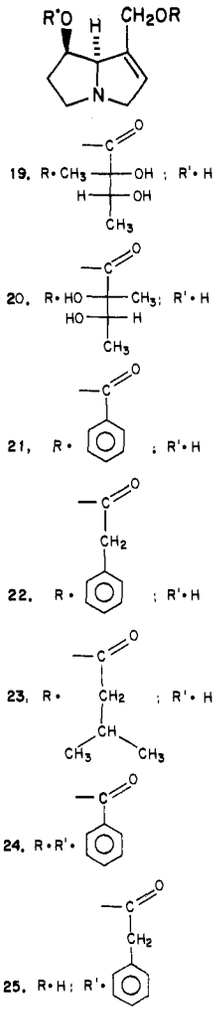
Culvenor and Smith[35] first showed that lycopsamine and intermedine could be distinguished by their C-methyl signals in the 60-MHz NMR spectra of mixtures, and recently Mohanraj and Herz[54] showed that viridiflorates and trachelanthates of saturated necines could be differentiated by means of the magnetically nonequivalent isopropyl methyl groups in C_6D_6 and by chemical shifts and patterns of the H-4′ and H-9 signals at 270 MHz. The isopropylidene derivatives, in fact, turn out to be very useful in distinguishing between these diastereomers when their NMR spectra are examined together with the parent compounds. Thus, the chemical shift position of the terminal methyl group of the acid side chain (C-4′) allows one to determine which family, the trachelanthic acid family (indicine **7** or intermedine **14**) or the viridifloric acid family (lycopsamine **15**[57] or unknown **16**), the alkaloid belongs to as follows: indicine (**7**), 1.15 d; intermedine (**14**), 1.19 d; lycopsamine (**15**), 1.25 d; and **16**, 1.25 d. For the corresponding isopropylidene derivatives the values are as follows: 1.43 d, 1.44 d, 1.29 d, and 1.25 d, respectively. Likewise, the chemical shift position of the C-3′ proton of the acid side chain in the isopropylidene derivatives can be used to distinguish the trachelanthates (4.29 and 4.32) from the viridiflorates (4.22 and 4.22). Whereas the chemical shift positions of the C-3′ protons in the parent alkaloids are similar for lycopsamine (**15**) (3.96 q), **16** (3.96 q), and indicine (**7**) (4.00 q), that for intermedine (**14**) (4.09 q) is clearly distinguishable. The same can be said for the isopropyl methine proton (C-5′) of intermedine (**14**) (2.03 hept), indicine (**7**) (2.13 hept), lycopsamine (**15**) (2.16 hept), and **16** (2.14 hept). Having decided which family the unknown alkaloid belongs to, an examination of the C-9 chemical shifts in the parent alkaloids and their isopropylidene derivatives permits an unequivocal structure assignment. Thus, for the parent alkaloids indicine (**7**) and intermedine (**14**) the C-9 shifts are very different: **7** (5.07 d, 4.57 d) and 14 (4.84 d, 4.75 d), whereas those of lycopsamine (**15**) (4.84 d, 4.73 d) and **16** (4.86 d, 4.71 d) are not very useful. Even in this case, an examination of the 3β and 5α chemical shifts in **15** (3β, 3.38 dd; 5α, 3.24 dd) and **16** (3β, 3.50 dd; 5α, 3.42 dd) allows a distinction. However, of greater utility was an examination of the C-9 chemical shifts in the protected esters of lycopsamine (4.74 d, 4.67 d), **16** (4.91 d, 4.57 d), indicine (4.80 d, 4.61 d), and intermedine (4.77 d, 4.63 d) in which case lycopsamine and **16** are readily distinguished. Thus, high-resolution NMR spectroscopy was used in this work to determine the optical purity of the coupled products.

For comparison purposes, the related nonnatural products **19** and **20** were prepared by coupling the racemic *threo*-2,3-dihydroxy-2-methylbutyric acids,[58] as their isopropylidene derivatives, to give the protected mixture of esters. The free mixture of diasteriomeric esters **19** and **20** were obtained, as previously described, by hydrolysis. In this case, the coupling of the racemic protected acid gave approximately a 1:1 mixture of diastereomeric protected esters. No attempt was made to resolve the acids prior to coupling, and the diastereomeric mixtures (esters and protected esters) were screened as their *N*-oxides, with interesting results, as described below. Finally the simple esters **21–23** were prepared for screening. While **21** has not previously been reported in the literature, the dibenzoate **24** was first reported as a synthetic product[60,61] and more recently as a natural product isolated from *Caccinia glauca*.[59] We observed the dibenzoate as a minor product in the preparation of **21** and converted **21** into the dibenzoate **24** for the purpose of identifying the minor product. In the preparation of the C-9 mono(phenylacetate) **22**, the C-7 mono(phenylacetate) **25** was isolated after chromatography, as a minor product, and its spectral properties are also included in the Experimental Section.



Biology. All of the compounds submitted for screening, except for **22** and **23**, were transformed into their water-soluble *N*-oxides by treatment of their chloroform solutions with *m*-chloroperbenzoic acid, followed by passage of gaseous ammonia through the solution to precipitate the acids. A short chromatography gave the *N*-oxides, which were characterized by ^{1}H NMR and TLC and quickly sealed under vacuum for submission for screening. The screening results are outlined in Table I. Our primary goal was to compare the compounds in question with indicine *N*-oxide and, in particular, to determine if any of the diastereomers (**14**, **15**, **16** *N*-oxides) or closely related isomers (**19**, **20** *N*-oxides) were more potent than indicine *N*-oxide. Also, included in Table I are some totally synthetic compounds (**21–23**, **17**, and **18**). Since all of the compounds were compared, side by side, with indicine *N*-oxide, the latter appears in Table I each time a group of compounds was screened. Indicine *N*-oxide, in every case, was screened at dose levels of 1600, 800, 400, and 200 mg/kg since it is not a particularly potent drug, whereas our initial synthesis

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Table I. Antitumor Activity in the P388 Lymphocytic Leukemia System[a]

	compd	dose/inj,[b] mg/kg	survivors, day 5	WGT diff (T - C)	% T/C
group I[c]	indicine	1600	06/06	-2.2	142
	N-oxide	800	06/06	-3.2	160
	(7 *N*-oxide)	400	06/06	-1.6	151
		200	06/06	-1.6	133
	21 *N*-oxide	70	06/06	-1.0	118
		35	06/06	-0.1	109
		17.5	06/06	-0.7	110
		8.75	06/06	-0.4	107
	intermedine	192	06/06	-1.1	109
	N-oxide	96	06/06	-0.1	107
	(14 *N*-oxide)	48	06/06	-0.4	105
		24	06/06	-0.1	100
	lycopsamine	192	06/06	-1.1	123
	N-oxide	96	06/06	-0.3	114
	(15 *N*-oxide)	48	06/06	-0.6	114
		24	06/06	-0.0	107
	16 *N*-oxide	192	06/06	-4.8	118
		96	05/06	0.1	116
		48	06/06	1.3	100
		24	06/06	-0.4	100
group II	indicine	1600	06/06	-3.7	117
	N-oxide	800	06/06	-3.3	200
	(7 *N*-oxide)	400	06/06	-1.9	145
		200	06/06	-0.5	120
	21 + 22	84	06/06	0.1	114
	N-oxide	42	06/06	0.3	117
		21	06/06	-0.1	108
		10.5	06/06	0.0	104
	19 + 20	68	06/06	-0.4	131
	N-oxide	34	06/06	-0.4	127
		17	06/06	0.0	117
		8.5	06/06	-0.3	115
group III	indicine	1600	05/06	-5.8	
	N-oxide	800	06/06	-4.7	200
	(7 *N*-oxide)	400	06/06	-4.3	191
		200	06/06	-2.9	170
	22	480	06/06	-2.1	116
		240	05/06	-0.1	105
		120	06/06	-0.6	109
		60	06/06	-0.3	97
		30	06/06	-0.5	99
		15	06/06	-0.8	97
	23	480	06/06	-2.7	114
		240	06/06	-0.8	108
		120	06/06	-0.1	108
		60	06/06	-0.4	105
		30	06/06	-0.3	109
		15	06/06	-0.6	105
group IV	indicine	1600	05/06	-2.9	
	N-oxide	800	06/06	-2.6	112
	(7 *N*-oxide)	400	06/06	-1.7	146
		200	06/06	-0.7	140
	17 + 18	300	06/06	-2.2	166
	N-oxides	150	06/06	-2.1	157
		75	06/06	-1.7	149
		37.5	06/06	-1.1	146

[a] Screening was carried out under the auspices of the National Cancer Institute. For detailed explanations of procedures and data, see Instruction 14, Screening Data Summary Interpretation and Outline of Current Screen Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD 20205. [b] Q01D × 9. Single dose for 9 days. [c] Within each group, the indicine *N*-oxide, indicated as the first entry, serves as the internal control.

of the diastereomers provided only sufficient material to screen at a maximum dose of ~200 mg/kg. Our intent was to resynthesize any of these diastereomers which appeared more potent than indicine *N*-oxide. As can be seen, none of the diastereomers (group I) appear to be more potent than indicine *N*-oxide and, indeed, do not appear to be more potent than the simple *N*-oxide C-9 monobenzoate of retronecine (**21**). Group II of Table I is of interest. Thus, the diastereomeric mixture of *N*-oxides of **19** and **20** appears to show promise with T/C = 131 at 68 mg/kg and T/C = 127 at 34 mg/kg. It may very well be that the potency of one of these isomers is even much greater. The screening results for the diastereomeric mixture of **19** and **20** were of sufficient interest that we were requested by NCI to supply an additional 500 mg to continue testing. It is also interesting to note that the corresponding isopropylidene derivatives appear to be inactive in terms of the assay. Group III is not particularly interesting since the free synthetic amines **22** and **23** do not show very good potency even at concentrations of 480 mg/kg. Finally, we have included in Table I, the screening data, presented here for the first time, for the mixture of synthetic *N*-oxides of **17** and **18** (group IV) whose synthesis has been previously reported.[28] These are clearly more potent than anything else in Table I.

Experimental Section

General Methods. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained by using either a Varian T-60 spectrometer or a Bruker WM-300 spectrometer equipped with an Aspect 2000 data system. Chemical shifts are reported relative to internal Me_4Si (δ 0) or $CHCl_3$ (δ 7.24). IR spectra were recorded on a Perkin-Elmer 299 or a Beckman IR 4240 spectrophotometer. Optical rotations were taken with a Perkin-Elmer 141 polarimeter or on a Bendex ETL-NPL automatic polarimeter type 143A. Mass spectra were obtained by using a Varian MAT 112S spectrometer interfaced with an SS200 data system. Melting points were taken on a Kofler hot stage and are uncorrected. Column chromatography was carried out with EM aluminum oxide 90 active, activity III, eluting with Baker HPLC toluene and methanol mixtures. TLC was performed on EM precoated aluminum oxide 150 F-254 plates or aluminum oxide 60 PF254 plates.

(±)-Viridifloric Acid. (±)-Viridifloric acid was synthesized by the hydroxylation of *trans*-α-isopropylcrotonic acid with tungsten trioxide and 30% hydrogen peroxide as previously described by Adams and Van Duuren.[32] Yields of 35–46% of recrystallized (ether–hexane) material of mp 149–151 °C were obtained: lit.[32] mp 150 °C; ^{1}H NMR ($CDCl_3$, CD_3OD) δ 0.92 (d, 6 H), 1.27 (d, 3 H), 2.12 (hept, 1 H), 4.02 (q, 1 H); EIMS, (*m/e*) (relative intensity) 41 (43), 43 (67), 45 (39), 56 (34), 85 (42), 103 (100), 118 (53); CIMS, *m/e* (relative intensity) 163 (M + 1, 18), 117 (100).

(±)-Trachelanthic Acid. (±)-Trachelanthic acid was prepared by the hydroxylation of *trans*-α-isopropylcrotonic acid with osmium tetraoxide in the presence of chloric acid according to the procedure of Kochetkov et al.[31] Yields of 85% of recrystallized (ether–hexane) material of mp 117–119 °C were obtained: lit.[31] mp 116–118 °C; ^{1}H NMR ($CDCl_3$, CD_3OD) δ 0.98 (d, 6 H), 1.24 (d, 3 H), 2.10 (hept, 1 H), 4.14 (q, 1 H); EIMS, *m/e* (relative intensity) 43 (59), 45 (34), 57 (35), 85 (42), 103 (100), 118 (73); CIMS, *m/e* (relative intensity) 163 (M + 1, 67), 117 (100).

(+)-Viridifloric Acid and (−)-Viridifloric Acid. An ethereal solution of 5.3 g of (±)-viridifloric acid was treated with 1.05 equiv of (+)-α-phenylethylamine. After 6 h, 3.8 g of crystalline salt was collected. The mother liquor was evaporated to dryness, acidified with 30% sulfuric acid, and extracted five times with ether. Evaporation of the ether gave 3.2 g of acid, which was dissolved in ether and treated with (−)-α-phenylethylamine, similarly, to give 3.2 g of salt. The mother liquor was again, as above, converted into free acid, which, as before, was treated with (+)-α-phenylethylamine, affording an additional 0.9 g of salt. An attempt to obtain a second crop of the salt with (−)-α-phenylethylamine did not yield any crystals.

The combined crop of salt from (+)-phenylethylamine was crystallized three times from ethanol to give 2.3 g of salt: mp 167–169 °C; $[\alpha]^{25}_D$ −12.2° (*c* 1, EtOH). This salt was dissolved in a small volume of 30% sulfuric acid, extracted five times with ether, and dried over sodium sulfate, and the ether was removed by evaporation to give 1.21 g of (+)-viridifloric acid: mp 119 °C; $[\alpha]^{25}_D$ +2.8° (*c* 1, H_2O) [lit.[31] mp 126–127 °C; lit.[31] $[\alpha]^{21}_D$ +1.97 (*c* 1, H_2O)]. The first report of natural (+)-viridifloric acid was

recorded recently[53] from the hydrolysis of coromandaline: mp 122–124 °C; $[\alpha]^{25}_D$ +3.1° (c 0.5, EtOH); ^{1}H NMR and mass spectra reported[53,54] similar to that recorded here for (±)-viridifloric acid.

The salt of (−)-α-phenylethylamine gave, after two recrystallizations, 1.8 g of crystals: mp 168–169 °C; $[\alpha]^{25}_D$ +10.0° (c 1, EtOH) [lit.[31] mp 158–159 °C, $[\alpha]^{21}_D$ +8.5° (c 1, EtOH)]. From this, as above, was obtained 0.90 g of (−)-viridifloric acid: mp 126–127 °C (lit.[31] mp 126–126.5 °C); $[\alpha]^{25}_D$ −2.7° (c 1, H_2O) [lit.[31] $[\alpha]^{21}_D$ −2.0° (c 1, H_2O)]. (−)-Viridifloric acid obtained from hydrolysis of lycopsamine[35] was reported to show mp 121.5–124 °C, undepressed on admixture with (−)-viridifloric acid.[52] Further recrystallization of this sample from ether/light petroleum followed by prolonged drying at 70 °C under vacuum gave a product of mp 137–138 °C with softening at 124 °C.[35] Crowley and Culvenor[52] state that the melting point of (−)-viridifloric acid is markedly influenced by minute amounts of tenacious impurities. (−)-Viridifloric acid from lycopsamine was reported[35] to show $[\alpha]^{20}_D$ −0.8° (c 1.52, H_2O), while that from hydrolysis of echinatine was reported[52] to show $[\alpha]^{20}_D$ −1.3° (water).

(+)-Trachelanthic Acid and (−)-Trachelanthic Acid. The resolution of (±)-trachelanthic acid (8.6 g) was performed similarly to that described above to yield 4.5 g of salt from (−)-α-phenylethylamine: mp 168–170 °C (lit.[31] mp 156–158 °C); $[\alpha]^{25}_D$ −9.0° (c 1, EtOH) [lit.[31] $[\alpha]^{22}_D$ −9.4° (c 1, EtOH). Hydrolysis gave 2.13 g of (+)-trachelanthic acid: mp 89–90 °C (lit.[31] mp 89–90 °C); $[\alpha]^{23}_D$ +2.15° (c 1.7, H_2O) [lit.[31] $[\alpha]^{24}_D$ 3.8° (c 1, H_2O)]. Hydrolysis of natural intermedine was reported[35] to yield (+)-trachelanthic acid with mp 92–93 °C, while hydrolysis of rinderine[55] was reported to yield (+)-trachelanthic acid with mp 91–93 °C. In neither of the latter two cases were optical rotations reported directly for the isolated trachelanthic acid. Culvenor[56] reported the isolation of (+)-trachelanthic acid from the hydrolysis of supinine with $[\alpha]^{18}_D$ +2.3° (c 1.7, EtOH) and mp 93–94 °C and showed that the specific rotation of (+)-trachelanthic acid in water decreased at increasing concentration!

The salt (3.8 g) from (+)-α-phenylethylamine showed mp 168–170 °C and $[\alpha]^{25}_D$ −11.6° (c 1, EtOH). From this was obtained 1.86 g of (−)-trachelanthic acid: mp 89–90 °C; $[\alpha]^{25}_D$ −2.15° (c 1.8, H_2O) [lit.[31] mp 90–91 °C; lit.[31] $[\alpha]^{22}_D$ −2.4° (c 1, H_2O)]. A recent report[53] of the isolation of (−)-trachelanthic acid from the hydrolysis of heliovincine gives mp 91–92 °C, $[\alpha]^{20}_D$ −1.9° (c 0.53, EtOH), and IR, ^{1}H NMR, and MS spectra similar to those obtained in the study. (−)-Trachelanthic acid isolated by hydrolysis of the first isolated indicine[17] was reported to show mp 94 °C and $[\alpha]_D$ −3.4° (no solvent or concentration indicated).

4-(1-Methylethyl)-2,2,5-trimethyl-1,3-dioxolane-4-carboxylic Acids. Isopropylidene Derivatives of (−)- and (+)-Viridifloric Acid and (−)- and (+)-Trachelanthic Acid. The isopropylidene derivatives of the necic acids were prepared by a procedure analogous to that described by Piper[37] for the preparation of the isopropylidene derivative of (−)-trachelanthic acid. Thus, 160 mg of the acid, dissolved in 1.6 mL of 2,2-dimethoxypropane, was treated with 25 μL of concentrated HCl. The reaction mixture was kept at 25 °C for 90 min. The brown solid resulting from removal of the solvent was recrystallized from ethyl ether to give yields in the range of 60–90%. The following properties were obtained for the isopropylidene derivatives.

4(*R*)-(1-Methylethyl)-2,2,5(*S*)-trimethyl-1,3-dioxolane-4-carboxylic acid ((−)-trachelanthic acid isopropylidene): mp 53–54 °C (lit.[37] mp 51–53 °C); $[\alpha]^{25}_D$ +34.8° (c 1, EtOH) [lit.[37] $[\alpha]^{25}_D$ +35.9 ± 0.5° (c 1, EtOH)].

4(*S*)-(1-Methylethyl)-2,2,5(*R*)-trimethyl-1,3-dioxolane-4-carboxylic acid ((+)-trachelanthic acid isopropylidene): mp 52–53 °C; $[\alpha]^{25}_D$ −27.3° (c 1, EtOH); ^{1}H NMR for both trachelanthic acid isopropylidenes ($CDCl_3$), δ 0.89 (d, 3 H), 1.00 (d, 3 H), 1.44 (s, 3 H), 1.52 (s, 3 H), 1.47 (d, 3 H), 2.18 (hept, 1 H), 4.33 (q, 1 H); MS for both trachelanthic acid isopropylidenes, EIMS, *m/e* (relative intensity) 59 (100), 71 (17), 83 (16), 99 (25), 101 (23), 127 (18), 157 (58), 187 (41); CIMS, 203 (M + 1, 100). Anal. ($C_{16}H_{18}O_4$) C, H.

4(*R*)-(1-Methylethyl)-2,2,5(*R*)-trimethyl-1,3-dioxolane-4-carboxylic acid ((+)-viridifloric acid isopropylidene): mp 63–65 °C; $[\alpha]^{25}_D$ −0.61° (c 1, EtOH).

4(*S*)-(1-Methylethyl)-2,2,5(*S*)-trimethyl-1,3-dioxolane-4-carboxylic acid ((−)-viridifloric acid isopropylidene): mp 63–64 °C; $[\alpha]^{25}_D$ 0.81° (c 1, EtOH); ^{1}H NMR for both viridifloric acid isopropylidenes ($CDCl_3$), δ 1.05 (d, 6 H), 1.40 (d, 3 H), 1.45 (s, 3 H), 1.60 (s, 3 H), 2.13 (hept, 1 H), 4.32 (q, 1 H); MS for both viridifloric acid isopropylidenes, EIMS, *m/e* (relative intensity) 59 (100), 71 (17), 83 (18), 99 (31), 104 (27), 127 (16), 157 (64), 158 (15), 187 (32), CIMS *m/e* (relative intensity) 203 (M + 1, 100). Anal. ($C_{10}H_{18}O_4$) C, H.

Coupling of the Isopropylidene Derivatives of (−)- and (+)-Viridifloric Acid and (−)- and (+)-Trachelanthic Acid with Retronecine. The following general procedure was used. One equivalent of the isopropylidene derivative of the necic acid and 1.1 equiv of CDI were dissolved in DMF (~50 mL/g of necic acid derivative), and the solution was allowed to stand 10–15 min. Then 1 equiv of imidazoylsodium and 1 equiv of retronecine were added, and the solution was kept at 25 °C for 24 h. Evaporation of the solvent in vacuo left an oily residue which was distributed between water and chloroform. Several chloroform extracts were combined and back-extracted with water until all traces (as detected by TLC) of retronecine and imidazole were removed. If necessary, the products were sometimes further purified by column chromatography on activity III alumina, eluting with toluene containing up to 1.5% methanol. Yields of 50–70% were repeatedly obtained. The following spectral data were obtained for the intermediate isopropylidene derivatives of **7**, **14**, **15**, and **16** respectively.

Isopropylidene derivative of indicine (7 isopropylidene): noncrystallizing gum; EIMS, *m/e* (relative intensity) 93 (46), 94 (32), 99 (16), 136 (32), 138 (100), 157 (31), 187 (5), 222 (2), 254 (3), 324 (3), 339 (1); ^{1}H NMR ($CDCl_3$) δ 0.81 (d, 3 H), 0.97 (d, 3 H), 1.37 (s, 3 H), 1.47 (s, 3 H), 1.43 (d, 3 H, C-4′), 1.97 (br, 2 H), 2.10 (hept, 1 H), 2.7 (br, 1 H), 3.22 (dd, 1 H), 3.38 (dd, 1 H), 3.88 (d, 1 H), 4.12 (1 H), 4.23 (1 H), 4.29 (q, 1 H, C-3′), 4.61 (d, 1 H, C-9), 4.80 (d, 1 H, C-9), 5.83 (s, 1 H, C-2).

Isopropylidene derivative of intermedine (14 isopropylidene): noncrystallizing gum; EIMS, *m/e* (relative intensity) 93 (68), 94 (44), 99 (15), 136 (29), 138 (100), 157 (24), 187 (1), 222 (1), 254 (1), 324 (1); ^{1}H NMR ($CDCl_3$) δ 0.85 (d, 3 H), 1.00 (d, 3 H), 1.33 (s, 3 H), 1.47 (s, 3 H), 1.44 (d, 3 H, C-4′), 2.00 (br, 2 H), 2.13 (hept, 1 H), 2.70 (br, 1 H), 3.25 (dd, 1 H), 3.40 (dd, 1 H), 3.91 (d, 1 H), 4.14 (1 H), 4.26 (1 H), 4.32 (q, 1 H, C-3′), 4.63 (d, 1 H, 9), 4.77 (d, 1 H, 9), 5.86 (br s, 1 H, 2).

Isopropylidene derivative of lycopsamine (15 isopropylidene): noncrystallizing gum; EIMS, *m/e* 93 (63), 94 (35), 99 (16), 136 (20), 137 (19), 138 (100), 157 (31), 187 (3), 254 (2), 295 (3), 324 (5), 339 (1); ^{1}H NMR δ 0.98 (d, 3 H), 1.01 (d, 3 H), 136 (s, 3 H), 1.50 (s, 3 H), 1.29 (d, 3 H, 4′), 1.95 (br, 2 H), 2.07 (hept, 1 H), 2.73 (br, 1 H), 3.25 (dd, 1 H), 3.39 (dd, 1 H), 3.92 (d, 1 H), 4.12 (1 H), 4.21 (1 H), 4.22 (q, 1 H, 3′), 4.67 (d, 1 H, 9), 4.74 (d, 1 H, 9), 5.90 (br s, 1 H, 2).

Isopropylidene derivative of 16: noncrystallizing gum; EIMS, *m/e* 93 (58), 94 (32), 99 (13), 136 (24), 137 (14), 138 (100), 157 (25), 187 (3), 254 (1), 295 (2), 324 (4); ^{1}H NMR δ 0.96 (d, 3 H), 0.98 (d, 3 H), 1.37 (s, 3 H), 1.57 (s, 3 H), 1.25 (d, 3 H, 4′), 2.00 (br, 2 H), 2.07 (hept, 1 H), 2.70 (br, 1 H), 3.24 (dd, 1 H), 3.37 (dd, 1 H), 3.91 (d, 1 H), 4.15 (1 H), 4.29 (1 H), 4.22 (q, 1 H, 3′), 4.57 (d, 1 H, 9), 4.91 (d, 1 H, 9), 5.87 (br s, 1 H, 2). Anal. ($C_{18}H_{29}NO_5 \cdot {}^1/_2H_2O$) C, H, N.

Indicine (7), Intermedine (14), Lycopsamine (15), and Isomer 16. A typical procedure for the conversion of the isopropylidene intermediates, mentioned above, to the parent alkaloids was as follows. The isopropylidene intermediates were taken up in an excess of 0.6 N HCl (5×) solution and this solution was allowed to stand at 25 °C for 24 h. The solution was then made alkaline by the addition of K_2CO_3 and finally extracted with chloroform. The chloroform solution, after drying over sodium sulfate, was passed through a short column of activity III Merck alumina. Evaporation of the chloroform eluent gave the desired alkaloid with the properties shown below.

Indicine (7). Isolated as a viscous gum shown to be homogeneous by TLC on aluminum oxide, eluting with toluene–methanol (9:1) and detection by iodine. Piper et al.[37] also reported isolation of synthetic labeled indicine as a gum, whereas a melting point of 97–98 °C has been reported[17] for indicine isolated from natural sources. For **7**: $[\alpha]^{20}_D$ +11.2° (c 1, EtOH) [lit.[17] $[\alpha]^{20}_D$ +22.3° (c 1.65, EtOH)] from *Heliotropium indicum*; no $[\alpha]$ reported by Piper;[37] ^{1}H NMR ($CDCl_3$) δ 0.90 (d, 3 H, 6′), 0.93 (d, 3 H, 6′), 1.15 (d, 3 H, 4′), 1.97 (m, 2 H, 6α and 6β), 2.13 (hept,

1 H, 5′), 2.7 (m, 1 H, 5β), 3.23 (dd, 1 H, 5α), 3.40 (dd, 1 H, 3β), 3.90 (d, 1 H, 3α), 4.00 (q, 1 H, 3′), 4.13 (br s, 1 H, 8α), 4.26 (br s, 1 H, 7α), 4.57 (d, 1 H, 9), 5.07 (d, 1 H, 9), 5.89 (s, 1 H, 2). This spectrum is consistent with that of Piper[37] run in Me_2SO-d_6. The ^{13}C NMR in $CDCl_3$ was identical with that reported recently by Jones et al.[49] Piper[37] reports the ^{13}C NMR in Me_2SO-d_6. EIMS, *m/e* (relative intensity) 43 (100), 45 (36), 57 (41), 67 (21), 80 (35), 85 (30), 93 (93), 94 (73), 103 (54), 118 (47), 120 (56), 136 (43), 138 (89), 139 (28), 156 (7), 254 (4), 299 (1).

Intermedine (14): mp 137–138 °C (lit.[40] mp 140–142 °C), isolated from *Conoclinium coelestinum* (*Eupatorium coelestinum*); $[\alpha]^{25}_D$ +5.0° (*c* 0.5, EtOH) [lit.[35] $[\alpha]^{20}_D$ +4.8° (*c* 2.39, EtOH), isolated from *Amsinckia intermedia*; lit.[40] $[\alpha]_D$ +7.8° (no temperature of concentration reported), isolated from *C. coelestinum*; lit.[47] mp 141–142 °C, $[\alpha]^{20}_D$ +9.8° (*c* 1.49, EtOH), crystallized from acetone after purification as borate complex]; ^1H NMR ($CDCl_3$) δ 0.91 (d, 3 H, 6′), 0.92 (d, 3 H, 6′), 1.19 (d, 3 H, 4′), 1.95 and 2.01 (m, 2 H, 6α and 6β), 2.03 (hept, 1 H, 5′), 2.70 (m, 1 H, 5β), 3.28 (dd, 1 H, 5α), 3.42 (dd, 1 H, 3β), 3.90 (d, 1 H, 3α), 4.09 (q, 1 H, 3′), 4.13 (br s, 1 H, 8α), 4.24 (br s, 1 H, 7α), 4.75 (d, 1 H, 9), 4.84 (d, 1 H, 9), 5.94 (s, 1 H, 2). This spectrum is consistent with the recently reported spectrum of Herz et al.[40] The ^{13}C NMR in $CDCl_3$ was identical with that reported by Jones et al.[49] EIMS, *m/e* (relative intensity) 53 (16), 67 (20), 80 (28), 93 (100), 94 (82), 120 (8), 138 (87), 139 (29), 156 (5), 255 (2), 299 (2).

Lycopsamine (15): isolated as a viscous gum homogeneous by TLC; $[\alpha]^{25}_D$ +1.2° (*c* 1.2, EtOH) [lit.[35] $[\alpha]^{20}_D$ +3.1° (*c* 5.98, EtOH), isolated as a pure gum by countercurrent extraction from *Amsinckia intermedia*; lit.[47] mp 132–134 °C; $[\alpha]^{20}_D$ +5.7° (*c* 0.89, EtOH) by crystallization from acetone after purification as borate complex]; ^1H NMR ($CDCl_3$) δ 0.86 (d, 3 H, 6′), 0.91 (d, 3 H, 6′), 1.25 (d, 3 H, 4′), 1.95 (m, 2 H, 6α and β), 2.16 (hept, 1 H, 5′), 2.70 (m, 1 H, 5β), 3.25 (dd, 1 H, 5α), 3.38 (dd, 1 H, 3β), 3.90 (d, 1 H, 3α), 3.96 (q, 1 H, 3′), 4.15 (br s, 1 H, 8α), 4.26 (br s, 1 H, 7α), 4.73 (d, 1 H, 9), 4.84 (d, 1 H, 9), 5.89 (s, 1 H, 2). This spectrum is consistent with but of higher resolution than those previously reported for lycopsamine.[35,47,57] The ^{13}C NMR spectrum in $CDCl_3$ was identical with that reported by Jones et al.[49] EIMS, *m/e* (relative intensity) 67 (17), 80 (22), 93 (80), 94 (71), 120 (12), 138 (100), 139 (35), 156 (9), 255 (2), 299 (3).

C-9 ester of retronecine with (+)-viridifloric acid (16): isolated as a noncrystallizing gum homogeneous by TLC; $[\alpha]^{25}_D$ +1.6° (*c* 1, EtOH); ^1H NMR ($CDCl_3$) δ 0.85 (d, 3 H, 6′), 0.91 (d, 3 H, 6′), 1.25 (d, 3 H, 4′), 1.95 (m, 2 H, 6α and 6β), 2.14 (hept, 1 H, 5′), 2.70 (m, 1 H, 5β), 3.42 (dd, 1 H, 5α), 3.50 (dd, 1 H, 3β), 3.90 (d, 1 H, 3α), 3.96 (q, 1 H, 3′), 4.15 (br s, 1 H, 8α), 4.26 (br s, 1 H, 7α), 4.71 (d, 1 H, 9), 4.86 (d, 1 H, 9), 5.90 (s, 1 H, 2); EIMS, *m/e* (relative intensity) 43 (100), 67 (31), 80 (36), 93 (82), 94 (71), 138 (52), 156(3), 170 (1), 212 (2), 256 (1), 290 (0.1); exact calcd for $C_{15}H_{25}NO_5$ 299.1734, found 299.1786. Anal. ($C_{15}H_{25}NO_5 \cdot {}^1/_4H_2O$) C, H, N.

***N*-Oxides of Indicine** (7), **Intermedine** (14), **Lycopsamine** (15), **and 16.** The *N*-oxides were prepared by the following modified procedure. One equivalent of the alkaloid in chloroform was treated with 1.5 equiv of *m*-chloroperbenzoic acid and the solution was allowed to stand at room temperature for 20 min. Then, excess gaseous ammonia was passed through the solution, and the resulting precipitated ammonium salts were removed by filtration. After concentration, the filtrate was passed through a short column of activity III alumina, eluting with 0–4% methanol in chloroform. In each case, isolated *N*-oxides were shown to be homogeneous and different from their parent alkaloids by TLC and by their ^1H NMR spectra. The *N*-oxides in chloroform solution were placed in vials and the solvent was removed under vacuum, leaving a glass in each case, and the vials were sealed and sent for screening.

Retronecine 9-(2′,3′-Dihydroxy-2′-methylbutyrate) (19, 20). Racemic *threo*-2,3-dihydroxy-2-methylbutyric acid was prepared by the hydroxylation of tiglic acid with tungsten trioxide and 30% hydrogen peroxide according to the procedure of Adams and Van Duuren[32] in 86% yield: mp 106–108 °C (lit.[58] mp 110–111 °C); ^1H NMR ($CDCl_3$, CD_3OD) δ 1.23 (d, 3 H), 1.55 (s, 3 H), 3.97 (q, 1 H); EIMS, *m/e* 43 (93), 45 (65), 72 (38), 89 (17), 90 (100), 119 (2); CIMS, 135 (M + 1, 100%).

Racemic *threo*-2,3-dihydroxy-2-methylbutyric acid was converted into its isopropylidene derivative as previously described in 85% yield: mp 65.5–67 °C; ^1H NMR ($CDCl_3$, CD_3OD) δ 1.35 (d, 3 H), 1.54 (s, 3 H), 1.44 (s, 3 H), 1.61 (s, 3 H), 4.14 (q, 1 H); EIMS, *m/e* (relative intensity) 59 (59), 71 (32), 99 (12), 129 (23), 159 (16); CIMS, *m/e* 175 (M + 1, 100). Anal. ($C_8H_{14}O_4$) C, H.

One equivalent of the above isopropylidene derivative and 1.1 equiv of CDI were dissolved in ethanol-free, dry chloroform. When CO_2 evolution ceased, 0.9 equiv of retronecine was added and the reaction mixture was allowed to stand at 45 °C for 20 h. The usual workup gave an 82% yield of a pure mixture of C-9 diastereomeric diastereomer), derivatives of **19** and **20**: mp 111–117 °C (from acetone); ^1H NMR ($CDCl_3$) δ 1.22 and 1.23 (d, 3 H, two diastereomers, 4′), 1.37 (s, 3 H, 7′), 1.47 (s, 3 H, 7′), 1.52 and 1.56 (s, 3 H, two diastereomers, 5′), 1.95 (br m, 2 H, 6), 2.70 (m, 1 H, 5β), 3.21 (t, 1 H, 5α), 3.38 (m, 1 H, 3β), 3.89 (d, 1 H, 3α), 4.04 (q, 1 H, 3′), 4.11 (br s, 1 H, 8α), 4.18 and 4.25 (br, 1 H, two diastereomers, 7), 4.62 and 4.82 (d, 2 H, C-9 protons of one diastereomer), 4.71 and 4.78 (d, 2 H, C-9 protons of one diastereomer), 5.86 (s, 1 H, 2); EIMS, *m/e* (relative intensity) 43 (17), 71 (12), 93 (58), 94 (38), 129 (24), 136 (24), 138 (100), 159 (4), 226 (1), 267 (3), 296 (7), 311 (0.5); CIMS, *m/e* (relative intensity) 312 (M + 1, 100). Anal. ($C_{16}H_{25}NO_5 \cdot {}^1/_4H_2O$) C, H, N.

The *N*-oxides of the above protected esters of **19** and **20** were prepared as previously described and the *N*-oxides were obtained as a noncrystalline glass, showing a single spot by TLC, with the following ^1H NMR ($CDCl_3$ + 5% CH_3OH): δ 1.15 (d, 3 H, 4′), 1.32 (s, 3 H, 7′), 1.40 (s, 3 H, 7′), 1.47 and 1.48 (s, 3 H, two diastereomers, 5′), 1.93 (br d, 1 H, 6β), 2.51 (m, 1 H, 6α), 3.61 (m, 2 H, 5), 3.98 (q, 1 H, 3′), 4.33 and 4.36 (AB q, 2 H, 3α and 3β), 4.50 (br s, 1 H, 8), 4.54 (br s, 1 H, 7), 4.74 (s, 2 H, 9), 5.66 (s, 1 H, 2).

The mixture of deprotected esters **19** and **20** was prepared from the protected esters as previously described to give the mixture of esters **19** and **20** as a noncrystalline glass which showed a single spot by TLC: ^1H NMR ($CDCl_3$) δ 1.13 (d, 3 H, 4′), 1.36 (s, 3 H, 5′), 1.90 (br m, 2 H, 6), 2.65 (m, 1 H, 5β), 3.18 (t, 1 H, 5α), 3.36 (dd, 1 H, 3β), 3.74 (q, 1 H, 3′), 3.87 (d, 1 H, 3α), 4.08 (m, 1 H, 8), 4.23 (m, 1 H, 7), 4.82 and 4.65 (d, C-9 of one diastereomer), 4.77 and 4.68 (d, C-9 of other diastereomer), 5.80 (s, 1 H, 2); EIMS, *m/e* (relative intensity) 43 (32), 80 (31), 93 (72), 120 (20), 138 (100), 156 (4), 227 (3), 254 (4), 271 (5); exact mass calcd for $C_{13}H_{21}NO_5$ 271.1420, found 271.1439. Anal. ($C_{13}H_{21}NO_5 \cdot {}^1/_2H_2O$) C, H, N.

The *N*-oxide of the above mixture of **19** and **20** was prepared as previously described to give a glassy substance, homogeneous by TLC with the following ^1H NMR ($CDCl_3$, CH_3OH): δ 1.15 and 1.13 (d, 3 H, C-4 diastereomers), 1.29 (s, 3 H, 5), 1.95 (m, 1 H, 6β), 2.54 (m, 1 H, 6α), 3.65 (q, 1 H, 3′), 4.33 (d, 1 H, 3β), 4.40 (d, 1 H, 3α), 4.61 (m, 1 H, 7), 4.84 and 4.76 (d, C-9 one diastereomer), 4.78 and 4.71 (d, C-9, the other diastereomer), 5.90 (s, 1 H, 2).

C-9 Monobenzoate of Retronecine (21). 1,1′-Cabonyldiimidazole (376 mg, 2.32 mmol) and benzoic acid (236 mg, 1.94 mmol) were dissolved in 25 mL of dry THF under a nitrogen atmosphere. After the mixture was stirred at room temperature for 1 h, 300 mg (1.94 mmol) of retronecine was added. After the mixture stood at room temperature for 16 h, the THF was removed and the residue was taken up in 25 mL of chloroform. The latter solution was washed three times with 20 mL of water and then dried over $MgSO_4$ and the solvent removed in vacuo to give 474 mg (94%) of a colorless oil shown to be homogeneous by TLC on alumina using benzene–methanol (9:1) and shown by NMR to be **23**: ^1H NMR ($CDCl_3$) δ 1.95 (m, 2 H, 6α and 6β), 2.66 (m, 1 H, 5β), 3.23 (m, 1 H, 5α), 3.41 (dd, 1 H, 3β), 3.91 (d, 1 H, 3α), 4.19 (br s, 1 H, 8), 4.29 (br s, 1 H, 7), 4.93 (br s, 2 H, 9), 5.88 (br s, 1 H, 2), 7.48 (m, 2 H), 8.01 (m, 2 H); EIMS, *m/e* (relative intensity) 83 (55), 85 (38), 93 (100), 94 (23), 105 (20), 126 (11), 136 (12), 137 (29), 138 (16), 154 (12); exact mass calcd for $C_{15}H_{17}NO_3$ 259.1208, found 259.1188. Anal. ($C_{15}H_{17}NO_3 \cdot {}^1/_5CHCl_3$) C, H, Cl.

The *N*-oxide of **21** was prepared as previously described for the other *N*-oxides. The dibenzoate was present in the original reaction mixture as a minor impurity and was identified by converting the monobenzoate to the dibenzoate with benzoyl chloride and pyridine. The dibenzoate is known both as a natural product, isolated from *Caccinia glauca*,[59] and as a synthetic material prepared by diesterification from retronecine.[60,61] We present here, for the first time, useful high-resolution ^1H NMR ($CDCl_3$) data for the dibenzoate: δ 2.17 (m, 2 H, 6α and 6β), 2.73

(m, 1 H, 5β), 3.35 (m, 1 H, 5α), 3.52 (dd, 1 H, 3β), 4.02 (d, 1 H, 3α), 4.47 (br s, 1 H, 8), 5.51 (br s, 1 H, 7), 4.91 and 4.87 (AB q, 2 H, 9), 5.94 (br s, 1 H, 2), 7.36, 7.46, 7.89 (aromatic).

C-9 Mono(phenylacetate) of Retronecine (22). Compound **22** was prepared as described above for **23** and the crude reaction mixture was chromatographed on activity III alumina. The major product, C-9 monoester **22**, was eluted in $CHCl_3$–CH_3OH (97:3). For **22**: ^1H NMR ($CDCl_3$) δ 1.85 (m, 2 H, 6α and 6β), 2.66 (m, 1 H, 5β), 3.16 (dd, 1 H, 3β), 3.32 (m, 1 H, 5α), 3.83 (d, 1 H, 3α), 4.06 (br s, 2 H, 7 and 8), 4.69 (br s, 2 H, 9), 5.68 (br s, 1 H, 2), 7.26 (m, aromatic), 3.62 (s, 2 H, 2'); EIMS, *m/e* (relative intensity) 53 (13), 55 (20), 57 (12), 60 (14), 66 (16), 67 (12), 68 (21), 69 (11), 70 (17), 80 (17), 81 (27), 82 (11), 92 (16), 94 (100), 95 (48), 96 (12), 136 (10), 137 (13), 138 (23), 139 (22); CIMS, *m/e* (relative intensity) 274 (M + 1, 33), 69 (100); exact mass calcd for $C_{16}H_{19}NO_3$ 273.1366, found 273.1379. Anal. ($C_{16}H_{19}NO_3 \cdot {}^1/_4H_2O$) C-7 H. For C-7 monoester **25**: mp 83–85 °C; ^1H NMR ($CDCl_3$) δ 2.05 (m, 2 H, 6α and 6β), 2.55 (q, 1 H, 5β), 3.14 (dm, 1 H, 5β), 3.28 (m, 1 H, 3β), 3.83 (d, 1 H, 3β), 3.93 (s, 2 H, 9), 4.23 (br s, 1 H, 8), 5.27 (q, 1 H, 7), 5.38 (d, 1 H, 2), 3.54 (s, 2 H, CH_2Ph), 7.2–7.3 (aromatic); EIMS, *m/e* (relative intensity) 68 (12), 80 (95), 81 (14), 91 (42), 93 (12), 94 (34), 106 (61), 111 (100), 120 (10), 123 (34), 124 (26), 136 (25), 137 (47), 255 (23), 273 (7); exact mass calcd for $C_{16}H_{19}NO_3$ 273.1366, found 273.1326.

C-9 Ester of Retronecine and Isovaleric Acid (23). Compound **23** was prepared as described above and the crude product was chromatographed on activity III alumina, and **25**, the major product, was eluted in chloroform: ^1H NMR ($CDCl_3$), run at 60 MHz, consistent with that of other C-9 monoesters run at 300 MHz mentioned above; EIMS, *m/e* (relative intensity) 41 (16), 80 (18), 93 (100), 94 (35), 135 (15), 137 (35), 138 (37), 155 (21), 239 (1); exact mass calcd for $C_{13}H_{21}NO_3$ 239.1522, found 239.1508. Anal. ($C_{13}H_{21}NO_3 \cdot {}^1/_2H_2O$) C, H.

Acknowledgment. This investigation was supported by the National Cancer Institute, National Institutes of Health (Grant ROI CA31490). We thank James McManus for development of the procedure for preparation of the *N*-oxides.

Registry No. **7**, 480-82-0; **7** (*N*-oxide), 41708-76-3; **7** (isopropylidene), 95363-32-9; **14**, 10285-06-0; **14** (isopropylidene), 95462-10-5; **14** (*N*-oxide), 95462-14-9; **15**, 10285-07-1; **15** (isopropylidene), 95462-11-6; **15** (*N*-oxide), 95462-15-0; **16**, 95462-13-8; **16** (isopropylidene), 95462-12-7; **16** (*N*-oxide), 95462-16-1; **19**, 95363-35-2; **19** (isopropylidene), 95363-33-0; **19** (isopropylidene *N*-oxide), 95363-34-1; **19** (*N*-oxide), 95363-36-3; **20**, 95462-18-3; **20** (isopropylidene), 95462-17-2; **20** (isopropylidene *N*-oxide), 95463-25-5; **20** (*N*-oxide), 95462-19-4; **21**, 95363-37-4; **21** (*N*-oxide), 6870-33-3; **22**, 95363-38-5; **23**, 95363-39-6; *trans*-α-isopropylcrotonic acid, 94773-28-1; tiglic acid, 80-59-1; (±)-*threo*-2,3-dihydroxy-2-methylbutyric acid, 40634-99-9; (±)-*threo*-2,3-dihydroxy-2-methylbutyric acid (isopropylene), 95363-40-9; (±)-viridifloric acid, 17132-45-5; (+)-viridifloric acid·(+)-phenylethylamine, 95363-29-4; (−)-viridifloric acid·(−)-phenylethylamine, 95363-30-7; (+)-trachelanthic acid·(−)-α-phenylethylamine, 23944-49-2; (−)-trachelanthic acid·(+)-α-phenylethylamine, 95363-31-8; (−)-trachelanthic acid (isopropylidene), 95462-07-0; (+)-trachelanthic acid (isopropylidene), 95462-08-1; (+)-viridifloric acid (isopropylidene), 81816-10-6; (−)-viridifloric acid (isopropylidene), 95462-09-2; (±)-trachelanthic acid, 23944-47-0; (+)-viridifloric acid, 17233-93-1; (−)-viridifloric acid, 17132-48-8; (+)-trachelanthic acid, 23944-48-1; (−)-trachelanthic acid, 23944-50-5; retronecine, 480-85-3; phenylacetic acid, 103-82-2.

Synthesis and Antidepressant Profiles of Phenyl-Substituted 2-Amino- and 2-[(Alkoxycarbonyl)amino]-1,4,5,6-tetrahydropyrimidines[1]

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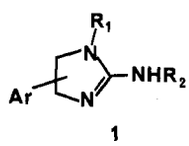
Received July 23, 1984

A series of 4(6)- and 5-phenyl-substituted 2-amino- and 2-[(alkoxycarbonyl)amino]-1,4,5,6-tetrahydropyrimidines **2** were prepared and evaluated for central nervous system (CNS) effects in animal models. Several 5-phenyl-substituted compounds possessed potent antidepressant activity and all compounds in this series were devoid of significant activity in any of the other CNS (anticonvulsant, muscle relaxant, and depressant) assays. The most active compound in the in vivo screen for antidepressant activity (reversal of reserpine-induced hypothermia), 2-[(methoxycarbonyl)amino]-5-phenyl-1,4,5,6-tetrahydropyrimidine (**16**), was considerably more potent than tricyclic antidepressant (TCA) standards. The 2-amino parent compound **27** on the other hand was >100-fold as effective as TCA's in in vitro inhibition of norepinephrine and dopamine uptake.

During our studies of structure–activity relationships (SAR) in a series of 2-[(alkoxycarbonyl)amino]-4(5)-phenyl-2-imidazolines **1**[2] (imidazolines) with central nervous system (CNS) activity we became interested in exploring the effect of enlarging of the imidazole moiety to give the appropriately substituted 2-amino-1,4,5,6-tetrahydropyrimidines **2** (tetrahydropyrimidines). A



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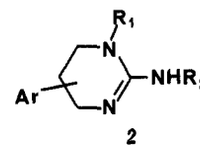

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R_1 = H or Me, R_2 = H or alkoxycarbonyl

number of representative compounds were prepared[3] and evaluated for their CNS activities[4] according to our previously reported procedures.[2]

Chemistry. Several methods were available for the preparation of cyclic guanidines from diamines and reactants containing the central guanidine C–N fragment. Therefore, our synthetic route was based in part on the intermediacy of 1-phenyl- and 2-phenyl-1,3-diaminopropanes (Scheme I). Cyanogen bromide was known to react with 1,2-diamines to yield 2-aminoimidazolines,[5] but it was not effective for the preparation of six- and seven-

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