Semisynthetic Pyrrolizidine Alkaloid *N*-Oxide Antitumor Agents. Esters of Heliotridine

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The C-9 and C-7 monoesters and C-7, C-9 diesters of heliotridine with (*S*)-(+)- and (*R*)-(−)-2-hydroxy-2-phenylbutyric acid were prepared, converted into their *N*-oxides, and compared with the corresponding C-9 monoesters of retronecine in the in vivo P388 lymphocytic leukemia screen. Relative in vitro cytotoxicities of some of the free bases and their corresponding *N*-oxides were also measured against the A204 rhabdomyosarcoma cell line by using the soft agar colony forming assay. Stereochemistry at C-7 of the necine and at C-2′ of the necic acid appears to have a significant effect on the antitumor activity in this system. In the heliotridine series, the configuration of the necic acid has a pronounced effect on the site selectivity (C-7 vs C-9) in esterification with carbodiimidazole. An explanation for this site selectivity is offered.

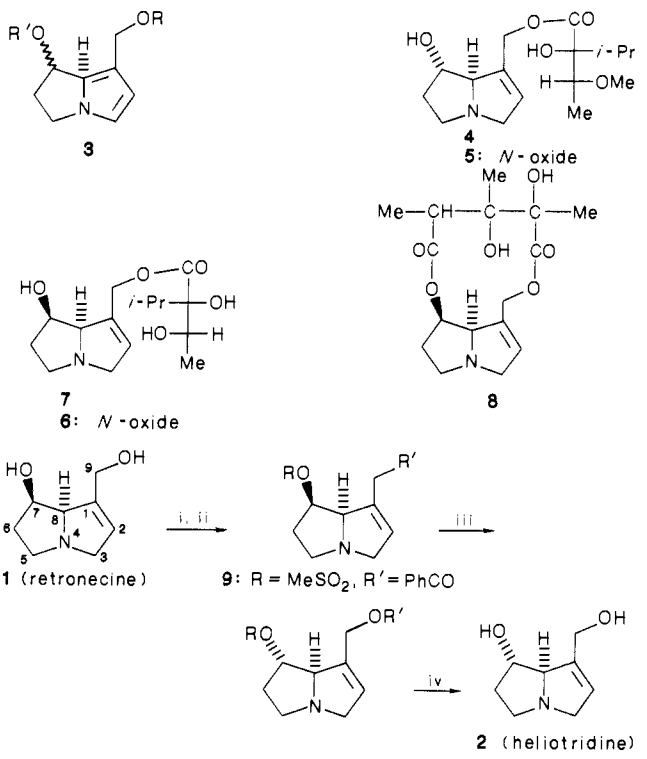
It has been reported that, on a molar basis, diesters of retronecine (**1**) and heliotridine (**2**) and about 4 times as toxic as the respective C-9 monoesters and heliotridine C-9 monoesters are 2–4 times as toxic as retronecine C-9 monoesters.[1] Thus, it was concluded that an α-OH at C-7 leads to higher hepatotoxicity than a β-OH. Metabolic pyrroles such as **3**, produced in the liver from pyrrolizidine alkaloids containing a double bond at C-1, have been identified as the cytotoxic agents.[2-4] More recently, the formation of *N*-oxides and pyrrolic intermediates, produced from unsaturated pyrrolizidine alkaloids by hepatic microsomal preparations, has been studied.[5] It was concluded that *N*-oxides and pyrroles are produced by independent pathways and acute pyrrolizidine hepatotoxicity was attributed only to the effects of the metabolic pyrroles. It has been suggested that pyrrolizidine *N*-oxides per se are not hepatotoxic, and their toxicity arises only to the extent that they are reduced to their corresponding bases.[6,7] A comparison of the toxicity of heliotrine (**4**), with its *N*-oxide (**5**), by intraperitoneal administration (ip) to the rat, showed acute LD_{50} of 300 mg/kg for the former and 5000 mg/kg for the latter.[8]

In 1976, the antitumor activity of indicine *N*-oxide (**6**) was discovered in a bioassay-directed fractionation of *Heliotropium indicum*.[9] Indicine *N*-oxide given ip is a more active antitumor agent than indicine (**7**) or heliotrine *N*-oxide (**5**) and indicine *N*-oxide administered orally is inactive.[10] Thus, indicine is not responsible for the antitumor activity of indicine *N*-oxide. The mechanism of the antitumor activity of indicine *N*-oxide is unclear at this time. As part of our continuing studies of the antitumor activity of semisynthetic pyrrolizidine alkaloid *N*-oxides,[11,12] we decided to examine the effect of stereochemistry at C-7 of the necine and at C-2′ of the necic acid moiety.

Chemistry

Our first concern was a ready supply of the necine, heliotridine (**2**). Although an elegant total synthesis of heliotridine has been reported, it did not lend itself to a practical solution to our problem.[13] Our earlier investigations of plants containing pyrrolizidine alkaloids bearing the necine heliotridine,[14] together with further attempts to find a ready source of this necine, failed to provide a ready supply of heliotridine. We were thus required to develop a practical conversion of retronecine into heliotridine. Retronecine was easily available by hydrolysis of monocrotaline (**8**), available in large quantities from the seeds of *Crotalaria spectabilis*.[11,12] Using the method of

Scheme I[a]



[a] (i) PhCOOH (1 equiv), CDI (1.1 equiv), THF, room temperature, 16 h, 95%. (ii) $MeSO_2Cl$ (1.3 equiv), Et_3N (1.5 equiv), CH_2Cl_2, −2 °C, 1.5 h, 93%. (iii) RCOOCs (4 equiv), DMF. (iv) $Ba(OH)_2$, room temperature, 87%.

Kellogg et al.,[15] we developed an efficient synthesis of heliotridine by nucleophilic displacement of the C-7 me-

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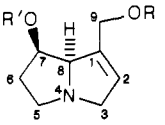
sylate, in the 7-mesyl-9-benzoate of retronecine (**9**) with various cesium carboxylates in DMF, followed by hydrolysis as outlined in Scheme I. A preliminary communication of this work has appeared.[16]

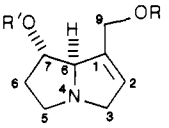
Our first choice as the synthetic necic acid, as previously mentioned,[11] was 2-hydroxy-2-phenylbutyric acid. We chose this acid because, like 2,3-dihydroxy-2-isopropylbutyric acid, the necic acid of indicine, the C-2′ position was chiral and contained a tertiary hydroxyl group and the addition of an aromatic ring at C-2′ permits a structure–activity study using various substituents on the aromatic ring. Also, 2-hydroxy-2-phenylbutyric acid had been resolved and was of known absolute configuration.[17] This turned out to be an excellent choice since this necic acid, even when used as the racemic mixture, and coupled to retronecine at C-9, produced a diastereomeric mixture (**10** + **11**) more potent and more active than indicine *N*-oxide.[12] In addition, we found a large difference in potency and activity between individual diastereomers, thus showing that chirality at C-2′, at least in this series, did indeed effect antitumor activity.[18] We, later in this paper, discuss these results together with the effect of stereochemistry at C-7 of the necine.

2-Hydroxy-2-phenylbutyric acid was prepared as previously described, but was resolved with (+)- and (−)-ephedrine rather than quinine as reported earlier.[11] Coupling of (*S*)-(+)- or (*R*)-(−)-2-hydroxy-2-phenylbutyric acid with retronecine using 1,1′-carbodiimidazole is highly site specific for the C-9 position, giving respectively **10** and **11** with no isolatable amounts of the C-7 isomers. In contrast, when heliotridine was treated with the *R* or *S* acid and 1,1′-carbodiimidazole in THF, a mixture of the C-7 and C-9 monoesters and the C-7, C-9 diesters was produced in each case. However, interestingly, the ratio of C-9 to C-7 monoesters was dependent on the chirality of the necic acid. Thus, with the *S* acid approximately 3 parts of the C-9 ester **12** was formed for every 1 part of the C-7 ester **13**. The diester **14** was formed as a minor product. On the other hand, when the *R* acid was used, the reverse was true; that is, the ratio of C-9 (**15**) to C-7 (**16**) monoester was approximately 1:2. Again, the diester (**17**) was a minor product.

In order to gain more insight into the site selectivity demonstrated by the enantiomeric acids, a series of experiments was run as follows. Under the same experimental conditions, heliotridine (**2**) was treated with 1 molar equiv of racemic 2-hydroxy-2-phenylbutyric acid and CDI, and the resulting product mixture was separated by preparative TLC into three bands of increasing polarity, namely, a band of diesters, a band of C-7 monoesters, and, finally, a band of C-9 monoesters. While it was not possible to separate mixtures of C-7 diastereomers such as **13** and **16** or mixtures of C-9 diastereomers such as **12** and **15**, it was a relatively easy matter to separate mixtures of C-9 and C-7 esters such as **12** and **13** or **15** and **16**. Thus, from



10: R = (*S*)-(+)-2-hydroxy-2-phenylbutyryl; R′ = H
11: R = (*R*)-(−)-2-hydroxy-2-phenylbutyryl; R′ = H
24: R = (*S*)-(+)-2-hydroxy-2-phenylpropionyl; R′ = H (atrolactic acid)
25: R = (*R*)-(−)-2-hydroxy-2-phenylpropionyl; R′ = H



12: R = (*S*)-(+)-2-hydroxy-2-phenylbutyryl; R′ = H
13: R = H; R′ = (*S*)-(+)-2-hydroxy-2-phenylbutyryl
14: R = R′ = (*S*)-(+)-2-hydroxy-2-phenylbutyryl
15: R = (*R*)-(−)-2-hydroxy-2-phenylbutyryl; R′ = H
16: R = H; R′ = (*R*)-(−)-2-hydroxy-2-phenylbutyryl
17: R = R′ = (*R*)-(−)-2-hydroxy-2-phenylbutyryl
18: R = (*S*)-(+)-2-hydroxy-2-phenylbutyryl; R′ = (*R*)-(−)-2-hydroxy-2-phenylbutyryl
19: R = (*R*)-(−)-2-hydroxy-2-phenylbutyryl; R′ = (*S*)-(+)-2-hydroxy-2-phenylbutyryl

the experiments with pure enantiomeric acids all four of the monoesters were obtained in pure form and by ^{1}H NMR analysis the compositions of the monoester mixtures were determined for the reaction with racemic acid. Similarly, pure diesters **14** and **17** were obtained from the reactions with the enantiomeric acids. The remaining two diesters, **18** and **19**, required as NMR references, were obtained from esterification of the pure monoesters with the enantiomeric acids. From NMR analysis of the products obtained from the reaction of heliotridine with racemic 2-hydroxy-2-phenylbutyric acid, it was observed that the four diesters **14**, **17**, **18**, and **19** were produced in about a 1:1:1:1 ratio, while the C-7 monoesters **13** and **16** were formed in a ratio of 1:2, respectively, and the C-9 monoesters **12** and **15** were formed in a ratio of 2:1, respectively. Thus, in both the reactions of heliotridine with the enantiomerically pure acids and with the racemic acid, the *S* acid was found to have a preference for the C-9 position, whereas the *R* acid had a preference for the C-7 position.

In another experiment, each of the pure monoesters was esterified with use of racemic acid, in the presence of CDI, and the mixture of diesters produced was analyzed by ^{1}H NMR. In each case, no site selectivity was observed. Thus, **12** gave equal amounts of **14** and **18**, **13** gave equal amounts of **14** and **19**, **15** gave equal amounts of **17** and **19**, and **16** gave equal amounts of **17** and **18**. It appeared that site selectivity was dependent on the availability of both hydroxyl groups in heliotridine. This experiment also demonstrated that under the esterification conditions, no exchange of acyl groups between C-9 and C-7 OH groups occurred.

Finally, the role of the hydroxyl group in the necic acid was investigated. Under similar conditions, heliotridine was treated with (*R*)-(−)- and (*S*)-(+)-2-phenylbutyric acid.[19,20] The *S* acid afforded the C-9 ester **20** and the C-7 ester **21** in a ratio of 3:1 while the *R* acid gave **22** and **23** in a ratio of 2:1, respectively. We do not think that any significance can be placed in the difference between these two ratios and conclude that, in this case, the two enantiomeric acids do not show any significant site selectivity.

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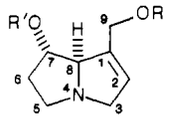
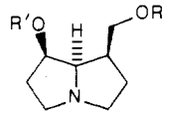
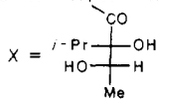


20: R = (*S*)-(+)-2-phenylbutyryl; R′ = H
21: R = H; R′ = (*S*)-(+)-2-phenylbutyryl
22: R = (*R*)-(−)-2-phenylbutyryl; R′ = H
23: R = H; R′ = (*R*)-(−)-2-phenylbutyryl
28: R = R′ = (*S*)-(+)-2-phenylbutyryl
29: R = R′ = (*R*)-(−)-2-phenylbutyryl



26: R = X; R′ = H
27: *N*-oxide of **26**
30: R = H; R′ = X



The observed site selectivity of (*R*)-(−)-2-hydroxy-2-phenylbutyric acid for the C-7 over the C-9 position of heliotridine must arise from diastereomerically different transition states. The critical intermediates leading to these transition states are the 1-acylimidazoles arising from the initial reactions of CDI with the acids. In Figure 1, we have attempted to illustrate how hydrogen bonding between (*R*)-(−)-(2-hydroxy-2-phenylbutyryl)imidazole and helliotridine places the C-7 hydroxyl group in a favorable position for nucleophilic attack on the acyl carbonyl group. We have recently completed the X-ray structures of (*S*)-(+)-2-hydroxy-2-(*p*-chlorophenyl)propionic acid and **11**. In each case, the intramolecular hydrogen bonding between the 2′ α-hydroxyl group and the ester or acid carbonyl could be seen, with the torsional angle O–C2′–C1′–O being 4.1° in the former case and 11.7° in the latter case.[18] Infrared evidence has also been presented to show intramolecular hydrogen bond in macrocyclic pyrrolizidine diester alkaloids.[21] Further intermolecular hydrogen bonding between the C-9 hydroxyl group of heliotridine and the N-3 of 1-acylimidazole would give the reactive intermediate illustrated in Figure 1. Such an intermediate would clearly be of higher energy for the (*S*-(+)-acylimidazole since the phenyl group would now suffer steric compression from the pyrrolizidine ring. In the case of retronecine, attack of the acyl carbonyl by the more reactive and less hindered C-9 hydroxyl group is energetically favored regardless of which enantiomeric acid is involved. Recent X-ray structures of retronecine and heliotridine[22] reveal, in the crystals, subtle conformational differences in the A ring. Thus the angle between the least-square planes defined by atoms C-1, C-2, C-3, N-4, C-8 and C-5, N-4, C-8, C-7 in retronecine was 124.4°, while in heliotridine it was 121.6°. In retronecine the left ring is exo puckered where the angle between C-5, C-6, C-7, and C-5, N-4, C-8 is 40.7°. In contrast, heliotridine is endo puckered where the corresponding angle is 42.2°.

In order to test the effect of the side chain, we initially prepared the racemic synthetic necic acids, and as previously mentioned, reaction of these acids with the more readily available retronecine in the presence of CDI gave, almost exclusively, the C-9 esters. Thus, the diastereomeric mixtures of **10** + **11** and **24** + **25** were prepared and screened as their *N*-oxides. ^{1}H NMR analysis indicated that the diastereomers, in each case, were produced in equal amounts. Also, in the former case, the acid was resolved to give, after coupling, the pure isomers **10** and **11**, which after conversion to their *N*-oxides were also screened in vivo. In the case of the heliotridine derivatives, only resolved necic acids were utilized in preparing samples for screening.

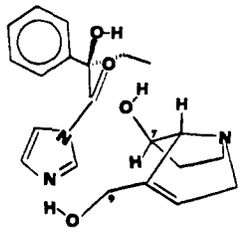


Figure 1.

Table I. Cytotoxicity against A204 Rhabdomyosarcoma Cell Line in Vitro[a]

compd	base IC_{50},[b] μg/mL	*N*-oxide IC_{50},[b] μg/mL	compd	base IC_{50},[b] μg/mL	*N*-oxide IC_{50},[b] μg/mL
10	>100	>100	**15**	>100	44 ± 2
11	>100	11 ± 0	**16**	>100	9 ± 1
12	>100	15 ± 3	indicine (and **6**)	34 ± 11	125 ± 22
13	>100	32 ± 1			
14	81 ± 1	ND	**26** (and **27**)	400 ± 140	320 ± 140
			8	316 ± 95	721 ± 38

[a] See the Experimental Section for details of preparation of soft agarose cultures. Cultures were conducted in quadruplicate to allow reliable estimates of the variance of the IC_{50} to be obtained. Control cultures with vehicle alone were always run at the same time. Dose–response curves were constructed with at least four drug concentrations to produce between 10 and 99.9% inhibition of cell growth. Dose–response curves were constructed on at least three different preparations. [b] To obtain the IC_{50}, the drug concentration producing 50% inhibition of cell growth, and its variance, the dose–response data was fitted to a monoexponential curve by using a NONLIN nonlinear least-squares regression analysis program. Variance of IC_{50} was obtained from the variance of the intercept and slope by using Taylor series expansion. Values are the mean ± SE. The highest concentration of the compound tested was 100 μg/mL, except for compounds **8**, **26**, **27**, indicine, and indicine *N*-oxide (**6**) where 1000 μg/mL was tested.

In order to gain insight into the mechanism of action of the antitumor activity of the pyrrolizidine alkaloid *N*-oxides and analogues, we required a dihydro *N*-oxide in large amount, in order to screen it at high enough dose levels to compare it with indicine *N*-oxide at its highest nontoxic dose. Because of these practical limitations we decided to prepare dihydroindicine *N*-oxide.[23] Indicine *N*-oxide was first reduced to indicine with zinc/acid and this in turn was hydrolyzed to retronecine and (−)-trachelanthic acid.[24] Retronecine was reduced with Raney nickel to give platynecine,[25] which was site selectively coupled with the acetonide of (−)-trachelanthic acid at C-9,[12] and finally the protecting group was removed to give dihydroindicine (**26**). The C-7 isomer, 7-(−)-trathelanthylplatynecine (**30**), was obtained as a minor product isolated from the mother liquor remaining after crystal-

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

compd no.	NSC no.	dose/inj,[b] mg/kg	survivors, day 5	wt diff, (T - C)	% T/C	compd no.	NSC no.	dose/inj,[b] mg/kg	survivors, day 5	wt diff, (T - C)	% T/C
12 *N*-oxide	377168	78	06/06	0.7	103	**(24 + 25)**	357486	200	06/06	-3.7	tox
		39	05/06	0.2	100	*N*-oxide		100	05/05	-2.2	198
		19.5	06/06	-0.5	106	repeat		50	06/06	-1.8	166
		9.75	06/06	1.2	106			25	06/06	-1.1	139
13 *N*-oxide	377167	102	05/05	0.1	97			12.5	06/06	-0.4	136
		51	06/06	0.2	103	10 *N*-oxide	369511	125	06/06	-1.3	125
		25.5	06/06	1.0	100			62.5	06/06	-0.8	114
		12.75	05/06	0.1	95			31.25	06/06	-0.7	110
14 *N*-oxide	377166	62	06/06	-0.2	127			15.63	06/06	-0.8	110
		31	06/06	0.1	109	11 *N*-oxide	369512	162	06/06	-3.4	214
		15.5	05/06	0.1	100			81	06/06	-3.2	169
		7.75	06/06	0.9	106			40.50	06/06	-2.4	151
15 *N*-oxide	377171	92	06/06	-0.5	126			20.25	06/06	-2.0	143
		46	05/06	1.8	117	6 reference	132319	1600	06/06	-2.2	142
		23	06/06	-0.1	106	for 10 + 11		800	06/06	-3.2	160
		11.5	06/06	-0.3	106			400	06/06	-1.6	151
16 *N*-oxide	377170	129	06/06	-0.4	106			200	06/06	-1.6	133
		64.5	05/06	0.1	106	**27 (26**	600090	1500	06/06	-0.5	106
		32.25	06/06	0.7	106	*N*-oxide)		750	06/06	-0.2	95
		16.13	06/06	-0.2	100			375	06/06	0.3	106
17 *N*-oxide	377169	71	05/06	-0.2	109			187.5	06/06	0.7	94
		35.5	06/06	-0.4	106	**26**	600089	1300	00/06	NA	toxic
		17.75	05/06	0.8	100			650	00/06	NA	toxic
		8.88	06/06	1.4	107			325	00/06	NA	toxic
(10 + 11) *N*-oxide	333058	300	06/06	-2.2	166			162.50	06/06	0.5	92
		150	06/06	-2.1	157	6 reference	132319	800	06/06	-1.5	146
		75	06/06	-1.7	149	for **26** + **27**		400	06/06	-1.1	120
		37.5	06/06	-1.1	146			200	05/06	0.0	118
6 reference for	132319	1600	05/06	-2.9	tox			100	06/06	0.5	101
(10 + 11)		400	06/06	-1.7	146	**30**	610331	92	06/06	0.6	98
N-oxide		200	06/06	-0.7	140			46	06/06	-0.6	105
(24 + 25)	357486	100	06/06	-3.6	180			23	06/06	-1.1	107
N-oxide[c]		50	06/06	-1.9	140			11.50	06/06	-1.2	111
		25	06/06	-1.3	96	**26** repeat	600089	325	00/06	NA	toxic
		12.5	06/06	-1.3	105			162.5	06/06	-0.9	98
		6.25	06/06	-0.4	105			81.25	06/06	0.0	102
6 reference for	132319	1600	06/06	-4.9	tox			40.60	06/06	-0.2	96
(24 + 25)		800	06/06	-3.6	196	6 reference	132319	800	04/04	-1.6	180
N-oxide		400	06/06	-2.6	175	for **30** +		400	04/04	0.1	123
		200	06/06	-2.1	152	**26** above		200	04/04	-0.6	126

[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] Q01Dx9. Single dose for 9 days. [c] Reference 18.

lization of dihydroindicine. Isomer **30** was obtained in pure crystalline form by use of dropping countercurrent chromatography. Dihydroindicine *N*-oxide (**27**) was prepared in the usual manner.

Biology

The relative in vitro cytotoxicities of some of the *N*-oxides and their bases were measured against the A204 human rhabdomysarcoma cell line by using the soft agar colony forming assay (Table I). For the retronecine derivatives **11** and the heliotridine derivatives **12**, **13**, **15**, and **16**, the *N*-oxides were more active than the bases. The retronecine derivative **10** was inactive, either as the base or the *N*-oxide. Indicine was more active than indicine *N*-oxide, but both were less active than the other compounds tested. Dihydroindicine and dihydroindicine *N*-oxide were relatively inactive and showed the same cytotoxicity. Monocrotaline was also relatively inactive in the in vitro assay but was more active than monocrotaline *N*-oxide. It is possible that some of the inactive compounds may also have shown activity if concentrations above 100 μg/mL were tested.

Table II shows the in vivo P388 lymphocytic leukemia screening data for the *N*-oxides of the heliotridine derivatives **12–17**, indicine *N*-oxide (**6**), and the *N*-oxides of retronecine derivatives **10**, **11**, diastereomeric mixture **10** + **11**, and diastereomeric mixture **24** + **25**. A comparison of the screening data for the heliotridine *N*-oxide derivatives of **12–17** with that for the retronecine *N*-oxide diastereomeric mixtures of **10** + **11** or **24** + **25** reveals that the stereochemistry of the C-7 hydroxyl group has an effect on potency. At comparable doses, the heliotridine derivatives are clearly less potent than the retronecine derivatives, and only **14** and **15** *N*-oxides appear to show activity at the doses measured. However, it should be pointed out that indicine *N*-oxide itself is not a very potent drug, and at the doses measured for heliotridine *N*-oxide derivatives of **12–17**, it also shows little activity. Thus, at higher doses these heliotridine derivatives might show activity comparable to that observed for indicine *N*-oxide.

A comparison of the T/C values for the retronecine *N*-oxide diastereomeric mixture of **10** + **11** with those for indicine *N*-oxide (**6**), run at the same time (compound **6** just below **10** + **11** in Table I), clearly reveals that the former are more potent than indicine *N*-oxide. Similarly, the analogous *N*-oxide diastereomeric mixture of **24** + **25** can be seen to be more potent than indicine *N*-oxide run at the same time. Table I includes a repeat of the screening of the *N*-oxide mixture of **24** + **25** run at another time, at higher dose level, and it can be seen that this mixture showed toxicity at 200 mg/kg, revealing that it is also more toxic than indicine *N*-oxide. The difference in the length of the alkyl side chains (Me vs Et) in the necic

acids of these retronecine derivatives does not appear to significantly effect the T/C values. The most dramatic data in Table I is seen for the C-9 retronecine *N*-oxide esters of 2-hydroxy-2-phenylbutyric acid; the ester of the *R* acid (**11** *N*-oxide) is far more potent and active than the ester of the *S* acid (**10** *N*-oxide), and the former is not only more potent but it is also more active than indicine *N*-oxide, showing a T/C of 214 at 162 mg/kg. This may not even be the best T/C value since the toxic dose was not reached in this experiment? A comparison of the screening results for the *N*-oxides of the C-9 heliotridine esters of **12** and **15**, run at the same time, shows that in this series also, the (*R*)-necic acid imparts more activity to the system than does the *S* acid. Comparison of **15** *N*-oxide with **11** *N*-oxide reveals that the stereochemistry of the C-7 hydroxyl group of the necine is significant in determining potency. This is also seen even when comparing isomers containing the less active (*S*)-necic acid in the retronecine *N*-oxide vs heliotridine *N*-oxide series (**10** *N*-oxide vs **12** *N*-oxide).

Comparing in vitro with in vivo results shows good correlations for the *N*-oxides of the retronecine derivatives. The retronecine *N*-oxide ester of *R*-(−)-2-hydroxy-2-phenylbutyric acid (**10**) shows high in vitro cytotoxicity and good in vivo antitumor activity, while the ester of (*S*)-(+)-2-hydroxy-2-phenylbutyric acid is much less active both in vitro and in vivo. Indicine *N*-oxide (**6**) shows moderate activity, but only at high doses in vivo and at high concentrations in vitro. Discrepancies exist between the in vivo and in vitro results for the *N*-oxides of the heliotridine derivatives. The derivatives **12** and **16** show high in vitro cytotoxicity but no activity in vivo, while the derivatives **13** and **15** show moderate in vitro cytotoxicity, but only **15** shows any activity in vitro, with derivative **13** being inactive. We assume that unfavorable pharmacokinetic factors account for the relative lack of activity of the heliotridine *N*-oxide derivatives in vivo compared to their in vitro cytotoxicity.

In this paper we have presented some structure–activity results, observed in the in vivo P388 lymphocytic leukemia antitumor screen, particularly regarding the effect of stereochemistry at the C-7 position of the necine in semisynthetic pyrrolizidine alkaloid *N*-oxides. The preliminary results shown here for the effect of stereochemistry in the necic acid portion of the drugs will appear in another forthcoming publication which will also describe the effects of various substituents in the necic acids. Ultimately, it is our goal to determine the mechanism of action of these compounds. In particular, we wish to determine how the *N*-oxides differ from the free bases in their selectivity for cancer cells and whether their toxicity is also mediated via pyrroles and whether such pyrroles, if they are the putative intermediates, are produced by prior reduction to the free bases or by direct formation from the *N*-oxides. In order to gain insight into the mechanism of action, we compared dihydroindicine *N*-oxide (**27**) with indicine *N*-oxide (**6**) in the screen. As can be seen in Table I, dihydroindicine *N*-oxide (**27**) is totally inactive in the screen when run at the same time as indicine *N*-oxide and run at a dose as high as 1500 mg/kg. We believe this result suggests that the antitumor activity of indicine *N*-oxide, and related semisynthetic pyrrolizidine alkaloid *N*-oxides, may also involve intermediate pyrroles. We are attempting to obtain experimental validation for this hypothesis.

Dihydroindicine (**26**) exhibited unexpected toxicity in the in vivo P388 lymphocytic leukemia system at doses of 1300–325 mg/kg. Since removal of the double bond from the necine portion of pyrrolizidine alkaloids results in loss of toxicity, it was thought that the toxicity of **26** was due to traces of the unnatural isomer **30** present as a contaminant. Therefore, the assay was repeated for **26**, **30**, and INO (**6**) as seen in the last three entries of Table II. While **26** still exhibited toxicity at 325 mg/kg, but not at lower doses, it is clear that the toxicity could not be due to traces of **30**, since the latter did not exhibit toxicity at any of the doses tested (92–11.50 mg/kg). The nature of this toxicity remains a mystery.

Experimental Section

In Vitro Cytotoxicity. Soft agarose cultures of A204 human rhabdomysarcoma cells were preformed as follows: Each 35-mm culture dish contained a base layer consisting of 0.5 mL of Dulbecco's modified Eagles medium containing 10% fetal calf serum with 0.5% agarose (growth media). On day 0 cells in bulk culture were dissociated with trypsin and EDTA, washed once in growth media, and subcultured by layering 10^4 viable cells in 0.5 mL of growth media with 0.3% agarose over each base layer. Cultures were examined with the aid of an inverted stage microscope and only cultures containing uniformly distributed single cell suspensions (< ten 30-μm cell cultures and no 60-μm clusters) were accepted for subsequent evaluation. Cultures were maintained in cell culture incubators at 37 °C, 5% CO_2–95% air, and 100% humidity. On day 1 (24 h later) an upper layer of 1 mL of growth media with and without the compound under investigation was added to the dishes. After 24 h, the upper layer of medium was removed by aspiration, and agarose culture surfaces were washed once with 0.5 mL of prewarmed growth media and then overlaid with 1 mL of fresh growth media. Colony formation was examined at daily intervals by conventional light microscopy. Cell lines form a sufficient number of detectable colonies (>60-μm diameter) for analysis following 7–9 days incubation. Viable colonies were stained with a metabolizable tetrazolium salt (2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride) and colonies counted with a Bausch & Lomb FAS-II image analysis system. Cultures were conducted in quadruplicate. Control cultures without drug were run at the same time.

General Methods. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in $CDCl_3$ on a Bruker WM 300 spectrometer equipped with an Aspect 2000 data system. Chemical shifts are reported relative to internal Me_4Si (δ 0.0). Optical rotations were measured on a Perkin-Elmer 141 polarimeter. 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 corrected. Analytical TLC was performed on EM precoated aluminum oxide 150 F-254 (type T) plates while preparative TLC was performed on 2-mm-thick plates of aluminum oxide (Merck type E 60 PF254).

(+)- and (−)-2-Hydroxy-2-phenylbutyric Acid. Racemic 2-hydroxy-2-phenylbutyric acid was prepared as previously described,[11] but was resolved by a different procedure using (+)- and (−)-ephedrine as described previously[12] for the resolutions of trachelanthic and viridifloric acids with (−)- and (+)-α-phenylethylamine. (*S*)-(+)-2-Hydroxy-2-phenylbutyric acid gave the less soluble salt with (−)-ephedrine, which, after three recrystallizations from ethyl ether containing 1–5% ethanol, showed mp 126–127 °C, $[\alpha]^{25}_D$ −4.8° (*c* 1, EtOH); (*R*)-(−)-2-hydroxy-2-phenylbutyric acid gave the less soluble salt with (+)-ephedrine, which after similar recrystallization, gave mp 127–128 °C, $[\alpha]^{25}_D$ +4.5° (*c* 1, EtOH). The salts were hydrolyzed with 6 M sulfuric acid and the acids were extracted into ethyl ether, evaporation of which gave the acids (*S*)-(+)-2-hydroxy-2-phenylbutyric acid, mp 129–130 °C, $[\alpha]^{24}_D$ +29.8° (*c* 1, EtOH) [lit.[11] mp 127–129 °C, $[\alpha]^{20}_D$ +29.0° (*c* 1.97, EtOH)] and (*R*)-(−)-2-hydroxy-2-phenylbutyric acid, mp 129–130 °C, $[\alpha]^{20}_D$ −28.1° (*c* 1, EtOH) [lit.[11] mp 119–124 °C, $[\alpha]^{24}_D$ −27.9°].

Heliotridine Esters of 2-Hydroxy-2-phenylbutyric Acid. Heliotridine was synthesized from retronecine as previously described.[16] To 55 mL of tetrahydrofuran was added 1.21 g (1 equiv) of (*S*)- or (*R*)-2-hydroxy-2-phenylbutyric acid and 1.20 g (1.15 equiv) of 1,1′-carbonyldiimidazole (CDI), and after 5 min, 1.04 g (1 equiv) of heliotridine was added to the solution. After the mixture was allowed to stand for 2 days at room temperature,

the solvent was removed in vacuo and the residue was partitioned between water and chloroform. The chloroform layer was washed with water, dried over sodium sulfate, and finally concentrated to dryness. From the *S* acid, 1.9 g of an ester mixture containing the 7,9-diester, the 7-monoester, and the 9-monoester was obtained, while the *R* acid yielded 1.6 g of a similar mixture.

The ester mixtures were separated by preparative TLC on 20 × 20 cm plates of aluminum oxide of the type previously described. Each plate was loaded with 190 mg of the reaction mixture, and the plates were developed with chloroform–acetone–methanol (47:47:6). The R_f values of the three bands, visualized with iodine vapor, were 0.71 for diesters **14** and **17**, 0.59 for C-7 monoesters **13** and **16**, and 0.29 for C-9 monoesters **12** and **15**, respectively. For the *S* acid, the ratio of C-9 to C-7 monoester was approximately 3:1, while the diester comprised about 7 molar % of the mixture. For the *R* acid the C-9 to C-7 monoester ratio was approximately 1:2 and the diester comprised about 14 molar % of the mixture. All of the esters were isolated in a pure form and characterized by ^{1}H NMR, MS, and elemental analysis.

Heliotridine Ester *N*-Oxides. The heliotridine esters were converted into the corresponding *N*-oxides as follows. To 300 mg of the ester in 40 mL of chloroform was added 300 mg of 85% *m*-chloroperbenzoic acid. After the mixture was allowed to stand at room temperature for 25 min, excess gaseous ammonia was passed through the solution, resulting in precipitation of the acids as their ammonium salts. The resulting slurry was cooled below 10 °C and filtered through Celite, and the filtrate was evaporated to yield the *N*-oxides as noncrystallizing gums, which were characterized by their 300-MHz ^{1}H NMR spectra, sealed under vacuum, and submitted for screening. The following properties were obtained for the various esters.

7,9-Di-*O*-[(*S*)-2-hydroxy-2-phenylbutyryl]heliotridine (**14**): noncrystallizing gum; ^{1}H NMR δ 0.86 (t, 3 H, C-4′), 0.91 (t, 3 H, H4′), 1.84 (m, 1 H, H6), 1.96 (m, 1 H, H6), 2.03 (m, 2 H, H3′), 2.18 (m, 2 H, H3′), 2.81 (m, 1 H, H5), 3.16 (m, 1 H, H5), 3.26 (dm, 1 H, H3), 3.86 (br s, 1 H, H8), 3.89 (br d, 1 H, H3), 4.69 & 4.74 (AB quartet, 2 H, H9), 4.99 (br s, 1 H, H7), 5.48 (s, 1 H, H2), 7.2–7.4 (m, 6 H), 7.5–7.6 (m, 4 H); EIMS, *m/e* (relative intensity) 43 (100), 58 (27), 77 (10), 105 (11), 119 (9), 135 (19), 300 (4); CIMS, *m/e* (relative intensity) 480 (M + 1, 100). Anal. ($C_{28}H_{33}NO_6 \cdot H_2O$) C, H, N.

7,9-Di-*O*-[(*S*)-2-hydroxy-2-phenylbutyryl]heliotridine *N*-oxide: noncrystallizing gum; ^{1}H NMR δ 0.83 (t, 3 H, H4′), 0.90 (t, 3 H, H4′), 2.0 (m, 1 H, H6), 2.03–2.15 (m, 4 H, H3′), 2.32 (m, 1 H, H6), 3.60 (m, 1 H, H5), 3.96 (m, 1 H, H5), 4.25 (br d, 1 H, H3), 4.46 (br d, 1 H, H3), 4.67 (s, 1 H, H8), 4.77 & 4.80 (AB quartet, 2 H, H9), 4.91 (s, 1 H, H7), 5.38 (s, 1 H, H2), 7.2–7.4 (m, 6 H), 7.45 (d, 2 H), 7.60 (d, 2 H).

7-*O*-[(*S*)-2-Hydroxy-2-phenylbutyryl]heliotridine (**13**): mp 92 °C; ^{1}H NMR δ 0.95 (t, 3 H, H4′), 1.95 (m, 2 H, H6), 2.03 (dq, 1 H, H3′), 2.20 (dq, 1 H, H3′), 2.84 (m, 1 H, H5), 3.10 (m, 1 H, H5), 3.28 (br d, 1 H, H3), 3.85 (s, 1 H, H8), 3.89 (br d, 1 H, H3), 4.18 (s, 2 H, H9), 5.10 (br s, 1 H, H7), 5.59 (s, 1 H, H2), 7.26 (t, 1 H), 7.33 (t, 2 H), 7.59 (d, 2 H); EIMS, *m/e* 43 (22), 57 (46), 71 (20), 80 (100), 94 (23), 106 (80), 111 (76), 120 (46), 137 (90), 165 (18), 270 (4), 317 (15); exact mass calcd for $C_{18}H_{23}NO_4$ 317.1627, found 317.1660. Anal. ($C_{18}H_{23}NO_4 \cdot {}^1/_4H_2O$) C, H, N.

7-*O*-[(*S*)-2-Hydroxy-2-phenylbutyryl]heliotridine *N*-oxide: noncrystallizing gum; ^{1}H NMR δ 0.84 (t, 3 H, H4′), 2.1 (m, 1 H, H6), 2.10 (dq, 1 H, H3′), 2.18 (dq, 1 H, H3′), 2.29 (m, 1 H, H6), 3.68 (m, 1 H, H5), 3.82 (m, 1 H, H5), 4.22 (s, 2 H, H9), 4.35 (br d, 1 H, H3), 4.51 (br d, 1 H, H3), 4.79 (s, 1 H, H8), 5.16 (br s, 1 H, H7), 5.68 (s, 1 H, H2), 7.28 (t, 1 H), 7.36 (t, 2 H), 7.56 (d, 2 H).

9-*O*-[(*S*)-2-Hydroxy-2-phenylbutyryl]heliotridine (**12**): noncrystallizing gum; ^{1}H NMR δ 0.97 (t, 3 H, H4′), 1.80 (m, 1 H, H6), 1.93 (m, 1 H, H6), 2.05 (dq, 1 H, H3′), 2.25 (dq, 1 H, H3′), 2.52 (ddd, 1 H, H5), 3.20 (m, 1 H, H5), 3.26 (m, 1 H, H3), 3.77 (br d, 1 H, H3), 3.85 (br s, 1 H, H8), 3.97 (q, 1 H, H7), 4.74 (d, 1 H, H9), 4.84 (d, 1 H, H9), 5.52 (br s, 1 H, H2), 7.27 (t, 1 H), 7.33 (t, 2 H), 7.56 (t, 2 H); EIMS, *m/e* 43 (35), 57 (51), 71 (36), 80 (84), 83 (100), 85 (67), 93 (65), 105 (20), 111 (46), 135 (52), 138 (72), 155 (13), 273 (2), 317 (1); exact mass calcd for $C_{18}H_{23}NO_4$ 317.1627, found 317.1600. Anal. ($C_{18}H_{23}NO_4$) C, H, N.

9-*O*-[(*S*)-2-Hydroxy-2-phenylbutyryl]heliotridine *N*-oxide: noncrystallizing gum; ^{1}H NMR δ 0.84 (t, 3 H, H4′), 1.88 (m, 1 H, H6), 2.14 (m, 1 H, H6), 2.06 & 2.15 (dq each, 2 H, H3′), 3.55 (m, 1 H, H5), 4.11 (m, 1 H, H5), 4.15 (s, 1 H, H7), 4.27 (br d, 1 H, H3), 4.51 (br d, 1 H, H3), 4.78 (br s, 2 H, H9), 4.97 (br s, 1 H, H8), 5.48 (s, 1 H, H2), 7.2–7.4 (m, 3 H), 7.56 (d, 2 H).

7,9-Di-*O*-[(*R*)-2-Hydroxy-2-phenylbutyryl]heliotridine (**17**): noncrystallizing gum; ^{1}H NMR δ 0.89 (t, 3 H, H4′), 0.91 (t, 3 H, H4′), 1.68 (m, 1 H, H6), 2.05 (m, 1 H, H6), 2.05 (m, 2 H, H3′), 2.20 (m, 2 H, H3′), 2.86 (m, 1 H, H5), 3.10 (m, 1 H, H5), 3.30 (dm, 1 H, H3), 3.93 (br d, 1 H, H3), 4.05 (br s, 1 H, H8), 4.77 & 4.91 (AB quartet, 2 H, H9), 4.93 (br s, 1 H, H7), 5.64 (s, 1 H, H2), 7.2–7.4 (m, 6 H), 7.56 (d, 4 H); EIMS, *m/e* 43 (18), 57 (98), 77 (26), 93 (50), 94 (39), 105 (26), 119 (100), 120 (71), 135 (68), 136 (39), 282 (9), 300 (60), 432 (0.4); CIMS, *m/e* 163 (100), 480 (M + 1, 4). Anal. ($C_{28}H_{33}NO_6 \cdot H_2O$) C, H, N.

7,9-Di-*O*-[(*R*)-2-Hydroxy-2-phenylbutyryl]heliotridine *N*-oxide: noncrystallizing gum; ^{1}H NMR δ 0.87 (t, 3 H, H4′), 0.89 (t, 3 H, H4′), 2.0 (m, 1 H, H6), 2.07 (m, 2 H, H3′), 2.18 (m, 2 H, H3′), 2.35 (m, 1 H, H6), 3.65 (m, 1 H, H5), 3.93 (m, 1 H, H5), 4.32 (d, 1 H, H3), 4.50 (d, 1 H, H3), 4.64 (s, 1 H, H8), 4.61 & 4.91 (AB quartet, 2 H, H9), 4.90 (s, 1 H, H7), 5.63 (s, 1 H, H2), 7.2–7.4 (m, 6 H), 7.50 (d, 2 H), 7.57 (d, 2 H).

7-*O*-[(*R*)-2-Hydroxy-2-phenylbutyryl]heliotridine (**16**): mp 98 °C; ^{1}H NMR δ 0.93 (t, 3 H, H4′), 1.86 (m, 2 H, H6), 2.05 (dq, 1 H, H3′), 2.29 (dq, 1 H, H3′), 2.86 (m, 1 H, H5), 3.14 (m, 1 H, H5), 3.31 (dm, 1 H, H3), 4.09 (dd, 1 H, H8), 3.92 (d, 1 H, H3), 4.29 (s, 2 H, H9), 5.07 (s, 1 H, H7), 5.63 (s, 1 H, H2), 7.2–7.4 (m, 3 H), 7.56 (d, 2 H); EIMS, *m/e* 43 (16), 57 (80), 77 (26), 80 (71), 105 (27), 106 (76), 111 (54), 120 (38), 135 (100), 137 (74), 165 (14), 270 (2), 317 (7); exact mass calcd for $C_{18}H_{23}NO_4$ 317.1627, found 317.1657. Anal. ($C_{18}H_{23}NO_4$) C, H, N.

7-*O*-[(*R*)-2-Hydroxy-2-phenylbutyryl]heliotridine *N*-oxide: noncrystallizing gum; ^{1}H NMR δ 0.88 (t, 3 H, H4′), 2.18 (m, 1 H, H6), 2.10 (dq, 1 H, H3′), 2.20 (dq, 1 H, H3′), 2.38 (m, 1 H, H6), 3.72 (m, 1 H, H5), 3.90 (m, 1 H, H5), 4.16 & 4.12 (AB quartet, 2 H, H9), 4.34 & 4.39 (AB quartet, 2 H, H3), 4.75 (s, 1 H, H8), 5.13 (br s, 1 H, H7), 5.66 (s, 1 H, H2), 7.28 (t, 1 H), 7.33 (t, 2 H), 7.58 (d, 2 H).

9-*O*-[(*R*)-2-Hydroxy-2-phenylbutyryl]heliotridine (**15**): noncrystallizing gum; ^{1}H NMR δ 0.92 (t, 3 H, H4′), 1.80 (m, 1 H, H6), 1.90 (m, 1 H, H6), 2.07 (dq, 1 H, H3′), 2.26 (dq, 1 H, H3′), 2.58 (m, 1 H, H5), 3.24 (m, 1 H, H5), 3.27 (m, 1 H, H3), 3.78 (br d, 1 H, H3), 3.88 (br s, 1 H, H8), 4.03 (m, 1 H, H7), 4.69 & 4.94 (AB quartet, 2 H, H9), 5.57 (s, 1 H, H2), 7.30 (t, 1 H), 7.34 (t, 2 H), 7.59 (t, 2 H); EIMS, *m/e* 57 (19), 68 (14), 80 (91), 93 (74), 94 (38), 111 (68), 135 (22), 138 (100), 155 (27), 273 (3); CIMS, *m/e* 138 (95), 156 (100), 318 (M + 1, 54). Anal. ($C_{18}H_{23}NO_4$) C, H, N.

9-*O*-[(*R*)-2-Hydroxy-2-phenylbutyryl]heliotridine *N*-oxide: noncrystallizing gum; ^{1}H NMR δ 0.84 (t, 3 H, H4′), 1.88 (m, 1 H, H6), 2.14 (m, 1 H, H6), 2.06 & 2.15 (dq each, 2 H, H3′), 3.55 (m, 1 H, H5), 4.11 (m, 1 H, H5), 4.15 (s, 1 H, H7), 4.27 (br d, 1 H, H3), 4.51 (br d, 1 H, H3), 4.78 (br s, 2 H, H9), 4.97 (br s, 1 H, H8), 5.48 (s, 1 H, H2), 7.26 (t, 1 H), 7.31 (t, 2 H), 7.55 (d, 2 H).

Mixed Diesters 18 and 19. Diester **18** was prepared by treating **12** with (*R*)-(−)-2-hydroxy-2-phenylbutyric acid under conditions analogous to those described above; similarly, **19** was prepared by the reaction of **15** with the corresponding *S*-(+) acid. These crude diesters were isolated by preparative TLC and their NMR spectra were utilized for analyses by comparing the portions of their NMR spectra which were different, such as the C-2, C-9, and C-7 proton areas.

(+)- and (−)-α-Phenylbutyric Acid. Racemic α-phenylbutyric acid was resolved by a procedure analogous to that of Pettersson[20] using (+)- and (−)-α-phenylethylamine except that only ethanol was used as the solvent. The salt of (+)-α-phenylethylamine and (−)-α-phenylbutyric acid showed mp 156–159 °C and $[\alpha]^{27}_D$ −7.3° (*c* 1, EtOH), while the salt of (−)-α-phenylethylamine and (+)-α-phenylbutyric acid gave mp 158–162 °C and $[\alpha]^{25}_D$ +12.3° (*c* 1, EtOH). The salts were hydrolyzed with 30% sulfuric acid and the acids were extracted into ethyl ether, evaporation of which gave the acids as oils, the former giving $[\alpha]^{25}_D$ −76.5° (*c* 1, EtOH) and the latter $[\alpha]^{25}_D$ +80.5° [lit.[20] $[\alpha]^{25}_D$ −78.5° and $[\alpha]^{25}_D$ +78.6°].

Heliotridine Esters of 2-Phenylbutyric Acid. The heliotridine esters of (+)- and (−)-2-phenylbutyric acid were obtained similarly to the esters of 2-hydroxy-2-phenylbutyric acid. The

reaction was carried out on a 1-mmol scale for 6 days at room temperature and then worked up in the usual manner, affording 246 mg of a mixture of (+)-2-phenylbutyric acid esters and 267 mg of a mixture of (−)-2-phenylbutyric acid esters. The mixtures of 7-mono-, 9-mono-, and 7,9-diester were separated by preparative TLC on alumina, eluting with toluene–methanol (9:1) with the order of elution 7,9-diester > 7-monoester > 9-monoester. For the *S*-(+) acid, the ratio of C-9 to C-7 monoester was approximately 3:1, while for the *R*-(−) acid the C-9 to C7 monoester ratio was approximately 2:1. The diester comprised about 7 molar % of the mixture in each case. All of the esters were isolated in a pure form and characterized by ^{1}H NMR and mass spectral analysis, including exact mass determination as indicated below.

7,9-Di-*O*-[(*S*)-2-phenylbutyryl]heliotridine (28): noncrystallizing gum; ^{1}H NMR δ 0.87 (t, 6 H, H4′), 1.84 (m, 1 H, H6), 2.07 (m, 1 H, H6), 1.80–2.07 (m, 4 H, H3′), 2.72 (m, 1 H, H5), 3.10 (m, 1 H, H5), 3.22 (m, 1 H, H3), 3.41 & 3.40 (2 t, 2 H, H2′), 3.91 (s, 1 H, H8), 3.87 (br d, 1 H, H3), 4.73 & 4.76 (AB quartet, 2 H, H9), 4.97 (s, 1 H, H7), 5.36 (s, 1 H, H2), 7.24 (br m, 10 H); EIMS, *m/e* 91 (88), 93 (49), 119 (100), 120 (55), 136 (40), 205 (12), 284 (99), 301 (8), 447 (4); exact mass calcd for $C_{28}H_{33}NO_4$ 447.2410, found 447.2396.

7,9-Di-*O*-[(*R*)-2-phenylbutyryl]heliotridine (29): noncrystallizing gum; ^{1}H NMR δ 0.88 (t, 6 H, H4′), 1.80 (m, 1 H, H6), 2.08 (m, 1 H, H6), 1.65–2.10 (m, 4 H, H3′), 2.68 (m, 1 H, H5), 3.05 (m, 1 H, H5), 3.24 (m, 1 H, H3), 3.41 & 3.46 (2 t, 2 H, H2′), 4.01 (s, 1 H, H8), 3.87 (br d, 1 H, H3), 4.79 & 4.70 (AB quartet, 2 H, H9), 4.94 (s, 1 H, H7), 5.49 (s, 1 H, H2), 7.26 (br m, 10 H); EIMS, *m/e* 91 (66), 93 (36), 119 (92), 120 (50), 136 (38), 205 (14), 283 (62), 284 (100), 301(8), 447 (4); exact mass calcd for $C_{28}H_{33}NO_4$ 447.2410, found 447.2387.

7-*O*-[(*S*)-2-Phenylbutyryl]heliotridine (21): noncrystallizing gum; ^{1}H NMR δ 0.88 (t, 3 H, H4′), 1.80 (m, 2 H, H3′), 1.81 (m, 1 H, H6), 2.06 (m, 1 H, H6), 2.78 (m, 1 H, H5), 3.00 (m, 1 H, H5), 3.26 (m, 1 H, H3), 3.44 (t, 1 H, H2′), 3.79 (br s, 1 H, H8), 3.85 (br d, 1 H, H3), 4.21 (s, 2 H, H9), 5.00 (s, 1 H, H7), 5.54 (s, 1 H, H2), 7.26 (m, 5 H); EIMS, *m/e* 56 (36), 71 (70), 80 (94), 106 (100), 111 (77), 124 (22), 137 (82), 173 (5), 205 (1), 283 (4), 301 (5); exact mass calcd for $C_{18}H_{23}NO_3$ 301.1678, found 301.1755.

7-*O*-[(*R*)-2-Phenylbutyryl]heliotridine (23): noncrystallizing gum; ^{1}H NMR δ 0.90 (t, 3 H, H4′), 1.82 (m, 3 H, H3′ & H6), 2.12 (m, 1 H, H6), 2.80 (m, 1 H, H5), 3.10 (m, 1 H, H5), 3.29 (m, 1 H, H3), 3.46 (t, 1 H, H2′), 4.01 (br s, 1 H, H8), 3.92 (br d, 1 H, H3), 4.30 (s, 2 H, H9), 5.00 (s, 1 H, H7), 5.59 (s, 1 H, H2), 7.26 (m, 5 H); EIMS, *m/e* 80 (84), 91 (44), 106 (100), 111 (65), 124 (22), 137 (72), 173 (1), 205 (1), 283 (2), 301 (3); exact mass calcd for $C_{18}H_{23}NO_3$ 301.1678, found 301.1676.

9-*O*-[(*S*)-2-Phenylbutyryl]heliotridine (20): noncrystallizing gum; ^{1}H NMR δ 0.89 (t, 3 H, H4′), 1.86 (m, 2 H, H3′), 1.77 (m, 1 H, H6), 2.10 (m, 1 H, H6), 2.56 (m, 1 H, H5), 3.20 (m, 1 H, H5), 3.23 (m, 1 H, H3), 3.48 (t, 1 H, H2′), 3.78 (br s, 1 H, H8), 3.81 (br d, 1 H, H3), 4.01 (q, 1 H, H7), 4.81 & 4.62 (AB quartet, 2 H, H9), 5.53 (s, 1 H, H2), 7.25 (m, 5 H); EIMS, *m/e* 43 (71), 56 (67), 71 (100), 89 (48), 93 (71), 138 (60), 173 (9), 257 (2), 301 (1); exact mass calcd for $C_{18}H_{23}NO_3$ 301.1678, found 301.1685.

9-*O*-[(*R*)-2-Phenylbutyryl]heliotridine (22): noncrystallizing gum; ^{1}H NMR δ 0.89 (t, 3 H, H4′), 1.84 (m, 2 H, H3′), 2.12 (m, 1 H, H6), 1.75 (m, 1 H, H6), 2.54 (m, 1 H, H5), 3.20 (m, 1 H, H5), 3.23 (m, 1 H, H3), 3.51 (t, 1 H, H2′), 3.79 (br s, 1 H, H8), 3.81 (br d, 1 H, H3), 3.93 (q, 1 H, H7), 4.85 & 4.61 (AB quartet, 2 H, H9), 5.55 (s, 1 H, H2), 7.24 (m, 5 H); EIMS, *m/e* 43 (24), 56 (20), 71 (28), 91 (40), 93 (100), 138 (81), 155 (5), 173 (1), 257 (2), 301 (1); exact mass calcd for $C_{18}H_{23}NO_3$ 301.1678, found 301.1667.

Dihydroindicine (26). A solution of 10 mmol (2.02 g) of (−)-trachelanthic acid acetonide[12] and 11 mmol (1.75 g) of CDI in 25 mL of freshly distilled DMF was warmed at 70 °C for 5 min, after which the evolution of CO_2 ceased. Then, a solution of 10 mmol (1.67 g) of platynecine[25] in 50 mL of DMF was added, followed by 0.8 g of sodium imidazole. The reaction mixture was heated at 73 °C for 19 h. DMF was stripped off and 50 mL of water was added to the residue, which was then extracted with $CHCl_3$. The combined $CHCl_3$ layer was washed with water and finally dried over Na_2SO_4. Evaporation left 2.9 g (85%) of a glassy residue. The NMR spectrum of this material was consistent with that expected for dihydroindicine acetonide (C-9 protons as ABX pattern at 4.62 & 4.55, isopropylidene methyls singlets at 1.51 & 1.41), but the NMR spectrum also showed traces of C-7 ester (<7%) by the presence of a signal at δ 5.4. This material was dissolved in 50 mL of 0.6 N HCl and the solution was kept at 25 °C for 22 h. The solution was then treated with an excess of $NaHCO_3$ and washed with 10 mL of $CHCl_3$. The aqueous layer was concentrated to 8 mL, treated with 1.5 mL of K_2CO_3, and concentrated in vacuo again to give a wet solid, which was extracted with $CHCl_3$–MeOH (97:3). On concentration, the solution afforded 2.4 g of a solid, which on recrystallization gave 1.8 g of dihydroindicine, which showed the following properties: mp 175 °C; ^{1}H NMR δ 0.92 (d, 3 H, H6′), 0.96 (d, 3 H, H6′), 1.20 (d, 3 H, H4′), 1.80 (m, 3 H), 1.99 (hept, 1 H, H5′), 2.64 (m, 1 H), 2.78 (m, 2 H), 3.08 (m, 1 H), 3.20 (t, 1 H), 3.29 (dd, 1 H), 4.07 (q, 1 H, H3′), 4.24 (s, 1 H, H7), 4.52 & 4.65 (ABX, 2 H, H9); EIMS, *m/e* 82 (100), 95 (48), 96 (30), 114 (16), 140 (42), 158 (37), 240 (4), 257 (2), 268 (2), 283 (2); CIMS 302 (M + 1, 100). Anal. ($C_{15}H_{27}NO_5$) C, H, N.

The mother liquor containing the C-7 ester was chromatographed with use of a Dropping Counter Current Chromatograph and the solvent system $CHCl_3$–C_6H_6–CH_3OH–H_2O (5:5:7:2) in the ascending mode of operation. By this method 160 mg of the C-7 ester, 7-(−)-trachelanthylplatynecine (**30**), was eluted first followed by 360 mg of the C-9 ester. 7-(−)-Trachelanthylplatynecine (**30**) showed the following properties: mp 187 °C; ^{1}H NMR δ 0.91 and 1.00 (2 d, 6 H, H6′), 1.24 (d, 3 H, H4′), 1.87 (m, 2 H), 1.95 (hept, 1 H, H5′), 2.10 (m, 2 H), 2.61 (m, 1 H), 2.75 (m, 2 H), 3.22 (dt, 1 H), 3.32 (m, 1 H), 3.48 (dd, 1 H), 3.78 (d, 2 H, H9), 4.10 (q, 1 H, H3′), 5.34 (s, 1 H, H7); EIMS, *m/e* 43 (100), 113 (20), 139 (81), 140 (35), 156 (34), 158 (24), 256 (3), 301 (0.4); exact mass calcd for $C_{15}H_{27}NO_5$ 301.2108, found 301.1940. Anal. ($C_{15}H_{27}NO_5$) C, H, N.

Dihydroindicine *N*-Oxide (27). A sample of 4.4 g of dihydroindicine was dissolved in a mixture of 47 mL of $CHCl_3$ and 3 mL of MeOH a treated with 5.2 g of *m*-chloroperbenzoic acid. After 1 h the solvent was removed and the residue was partitioned between E_2O (40 mL) and H_2O (40 mL). The aqueous layer was separated and washed three times with 40 mL of Et_2O. Evaporation and drying gave 4.4 g of dihydroindicine *N*-oxide: mp 165–167 °C; ^{1}H NMR δ 0.92 (d, 3 H, H6′), 0.98 (d, 3 H, H6′), 1.21 (d, 3 H, H4′), 1.92 (hept, 1 H, H5′), 2.00 (m, 2 H), 2.32 (m, 1 H), 2.58 (m, 1 H), 3.22 (m, 1 H), 3.62 (m, 2 H), 3.77 (dd, 1 H), 3.85 (m, 2 H), 4.10 (q, 1 H, H3′), 4.62 (t, 1 H, H7), 4.40 & 4.74 (ABX, 2 H, H9). Anal. ($C_{15}H_{27}NO_6$) C, H, N.

Acknowledgment. This investigation was supported by the National Cancer Institute, National Institutes of Health (Grant ROI CA31490).

Registry No. **2**, 520-63-8; **6**, 41708-76-3; **10**, 81340-08-1; **10** (*N*-oxide), 114489-11-1; **11**, 81370-87-8; **11** (*N*-oxide), 114489-12-2; **12**, 114489-01-9; **12** (*N*-oxide), 114489-09-7; **13**, 114422-98-9; **13** (*N*-oxide), 114423-09-5; **14**, 114422-99-0; **14** (*N*-oxide), 114423-10-8; **15**, 114489-02-0; **15** (*N*-oxide), 114489-10-0; **16**, 114423-00-6; **16** (*N*-oxide), 114423-11-9; **17**, 114489-03-1; **17** (*N*-oxide), 114528-94-8; **18**, 114489-04-2; **19**, 114489-05-3; **20**, 114423-01-7; **21**, 114423-02-8; **22**, 114489-06-4; **23**, 114423-03-9; **24**, 114423-04-0; **24** (*N*-oxide), 114423-12-0; **25**, 114489-07-5; **25** (*N*-oxide), 114528-95-9; **26**, 114423-05-1; **26** (acetonide), 114423-14-2; **27**, 114423-06-2; **28**, 114423-07-3; **29**, 114489-08-6; **30**, 114423-08-4; (±)-$C_2H_5C(OH)C_6H_5CO_2H$, 81801-80-1; (*R*)-$C_2H_5C(OH)C_6H_5CO_2H$, 3966-31-2; (*S*)-$C_2H_5C(OH)C_6H_5CO_2H$, 24256-91-5; (*S*)-$C_2H_5C(OH)C_6H_5CO_2$·(−)-ephedrine, 114423-15-3; (*R*)-$C_2H_5C(OH)C_6H_5CO_2H$·(+)-ephedrine, 114423-13-1; (±)-$C_2H_5CH(C_6H_5)CO_2H$, 7782-29-8; (*R*)-$C_2H_5CH(C_6H_5)CO_2H$, 938-79-4; (*S*)-$C_2H_5CH(C_6H_5)CO_2H$, 4286-15-1; (*R*)-$C_2H_5CH(C_6H_5)CO_2H$·(*R*)-$C_6H_5CH(CH_3)NH_2$, 109640-25-7; (*S*)-$C_2H_5CH(C_6H_5)CO_2H$·(*S*)-$C_6H_5CHCCH_3NH_2$, 1349-02-6; (−)-trachelanthic acid acetonide, 95462-07-0; platynecine, 520-62-7.