A Study of Some Leukotriene A_4 and D_4 Analogues by Proton Nuclear Magnetic Resonance Spectroscopy

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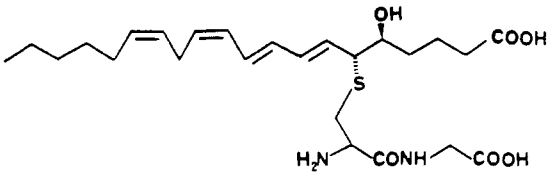
By use of a combination of homonuclear proton decoupling and iterative spectral simulation techniques, a detailed analysis of the proton NMR spectra of several protected analogues of leukotrienes A_4 and D_4 has been performed. The leukotriene D_4 analogues are shown to contain a rigid olefinic side chain which exhibits strong conformational preferences about the 5,6 and 6,7 bonds, causing the cysteine sulfur atom, linking the peptide side chain, to adopt a well-defined orientation about the 6,7 bond. The remainder of the molecule, consisting of alkyl ester and amino acid side chains, shows a high degree of conformational flexibility with several significantly occupied conformations. Studies carried out on the naturally occurring 5(*S*),6(*R*) isomer and its 5(*R*),6(*S*) analogue in the deprotected form in methanol-d_4 solution indicate a similarity in the relative conformational stabilities of the deprotected and protected forms of these compounds.

The leukotrienes are currently the center of considerable pharmacological[1-3] and synthetic[4-7] interest owing to their apparent mediating role in allergic asthma.[8] A series of compounds related to leukotriene D_4 (I) has recently been



I

synthesized in our laboratories[9] and the combined application of selective homonuclear decoupling and iterative spectral simulation used to perform a detailed analysis of their proton NMR spectra.

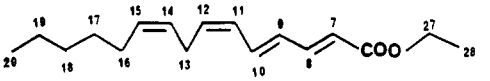
Experimental Section

Spectra were recorded at 250.13 MHz by using a Bruker WM-250 spectrometer with a probe temperature of ca. 29 °C. Samples consisted of 10–15 mg of compound in 1 mL of deuterated solvent (chloroform or benzene) and were degassed by using the freeze–pump–thaw technique prior to sealing, under vacuum, in 5-mm NMR tubes containing ca. 1% Me_4Si as an internal reference. Iterative spectral analysis was performed by using the standard Bruker PANIC program.

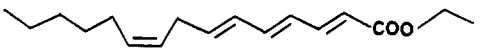
Results

The first detailed spectral assignments were performed on a series of ethyl esters, compounds II and III, which were intermediates in the synthesis of I.

Compound II gave a well-dispersed spectrum in which selective proton-decoupling techniques could be employed to distinguish, unambiguously, between all resonances other than the signals corresponding to the high-field al-



II

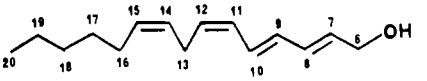


III

iphatic methylenes at positions 17–19.

With 26 individual protons, this molecule is too large for conventional spectral simulation techniques, and so the spin system was split by selectively decoupling the diallylic methylene at position 13. This leaves an olefinic system comprising six protons (positions 7–12) which can then be analyzed independently. Decoupling at 13 also caused the collapse of the complex multiplet formed by 14 and 15, permitting a direct determination of the coupling between these two nuclei. The remaining parameters were obtained on the basis of a first-order analysis, and the results obtained are listed in Table I.

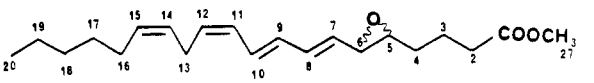
Reduction of ester II (the isomer corresponding to naturally occurring leukotrienes) yielded alcohol IV.[9] The



IV

experimentally observed and theoretically modeled spectra of the olefinic region of IV are shown in Figure 1. Note that the modeling of 12 is complicated by the overlap of signals corresponding to 14 and 15. Also, the signal at δ 6.31 due to 8 clearly shows the presence of a four-bond coupling of 1.6 Hz to the two methylene protons at 6 although, in this spectrum, the fine splitting at 6 is masked by the broadening caused by interaction with the exchangeable proton of the alcohol group.

The values obtained in these simpler precursor systems facilitated the analysis of (±)-LTA_4 methyl ester (V) and



V, (±)-LTA_4 methyl ester
VI, (±)-5,6-*cis*-LTA_4 methyl ester

its isomer with the alternative *cis*-epoxide ring geometry (VI). The two isomers were readily distinguished via the coupling between the epoxide methine protons, the trans isomer giving rise to a coupling of 1.8 Hz and the corre-

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Table I. Proton Chemical Shifts and Coupling Constants for LTA Analogues and Precursors

	chemical shift, δ						coupling constants, Hz				
group	II[a]	III[a]	IV[a]	V[b]	VI[b]	J	II[a]	III[a]	IV[a]	V[b]	VI[b]
2				2.07	2.07	2,3				7.3	7.4
3				1.30	1.30	3,4				7.3	7.4
4				1.60	1.59	4,5				3.9	5.3
5				2.76	2.54	5,6				4.3	1.8
6			4.20[c]	3.19	2.91	6,7			5.5[c]	7.1	7.4
						6,8			1.6[c]		
7	5.87[c]	5.87	5.86[c]	5.4-5.5	5.32	7,8	15.0[c]	14.9	14.2[c]	15.3	15.3
8	7.34[c]	7.32	6.31[c]	6.37	6.34	8,9	11.4[c]	10.2	10.5[c]	11.8	11.8
9	6.30[c]	6.25	6.23[c]	6.11	6.11	9,10	14.7[c]	14.9	14.2[c]	15.3	15.3
10	6.86[c]	6.55	6.51[c]	6.55	6.55	10,11	11.3[c]	10.2	11.4[c]	10.2	10.2
11	6.08[c]	6.19	6.03[c]	6.03	6.05	11,12	11.1[c]	14.9	11.5[c]	11.4	11.4
12	5.63[c]	5.93	5.30-5.48	5.4-5.5	5.4-5.5	12,13		6.4		6.3[d]	6.9[d]
13	2.97	2.90	2.92	2.93	2.94	13,14				6.3[d]	6.9[d]
14	5.34	5.40	5.30-5.48	5.4-5.5	5.4-5.5	14,15		10.0			
15	5.42	5.51	5.30-5.48	5.4-5.5	5.4-5.5	15,16					
16	2.04	2.03	2.06	2.02	2.03	19,20	7.1	6.8		6.3	6.3
17	1.12-1.39	1.24-1.35	1.2-1.4	1.2-1.3	1.2-1.3	27,28	7.1	7.0			
18	1.12-1.39	1.24-1.35	1.2-1.4	1.2-1.3	1.2-1.3						
19	1.12-1.39	1.24-1.35	1.2-1.4	1.2-1.3	1.2-1.3						
20	0.88	0.89	0.88	0.88	0.88						
27	4.19	4.21		3.32	3.33						
28	1.28	1.30									

[a] Sample consists of ca. 15 mg of compound in 1.0 mL of $CDCl_3$. [b] Sample consists of ca. 15 mg of compound in 1.0 mL of C_6D_6. [c] Iterated second-order value. [d] Averaged value.

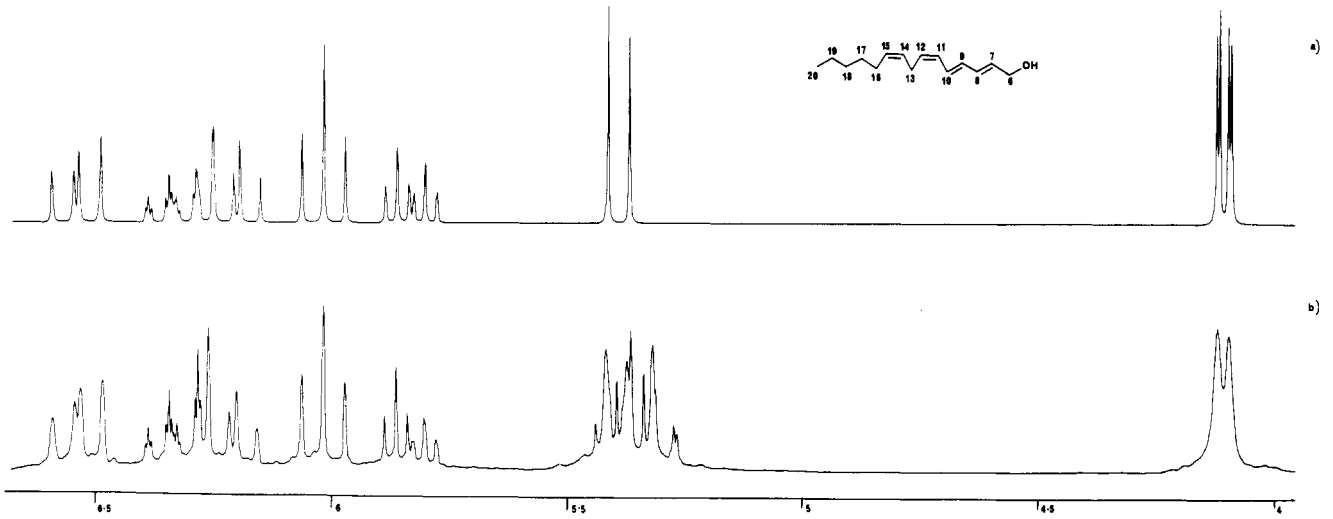
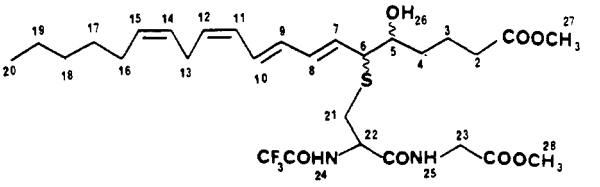


Figure 1. (a) Theoretical spectrum of the olefin proton signals of IV; (b) 250-MHz proton spectrum of the olefinic spectrum of IV in deuteriochloroform solution.

sponding cis isomer giving 4.3 Hz. In each case, this value was obtained by selectively decoupling the adjacent methylene protons at 4, leaving a distinct doublet signal for 5. The complete values for these two compounds are given in Table I.

The chemical shift values obtained for many of the high-field aliphatic signals differ appreciably from those reported in a study by Baker et al.,[10] and this is believed to be directly attributable to the effect of the benzene-d_6 solvent. An additional study which we carried out in deuteriochloroform solution gave results which were very similar to those reported by Baker, and, although explicit mention of the solvent was not made in his paper, it would appear likely that deuteriochloroform was used.

The leukotriene D_4 analogues were obtained, as reported,[9] by addition of *N*-(trifluoroacetyl)cysteinylglycyl methyl ester to the isomerically pure (±)-*cis*- and (±)-*trans*-epoxides V and VI. The resulting pairs of protected leukotriene D_4 diastereomers were separated by HPLC and their stereochemistries assigned as reported previously.[9] Naturally occurring leukotriene D_4 possesses the 5(*S*),6(*R*) configuration and corresponds directly with VIID minus the two methyl and trifluoroacetyl protecting groups.



form	configuration
VIIA	5(*R*),6(*R*)
VIIB	5(*S*),6(*S*)
VIIC	5(*R*),6(*S*)
VIID	5(*S*),6(*R*)

The spectrum of VIIA in benzene-d_6 solution is shown in Figure 2. As can be clearly seen, despite its overall level

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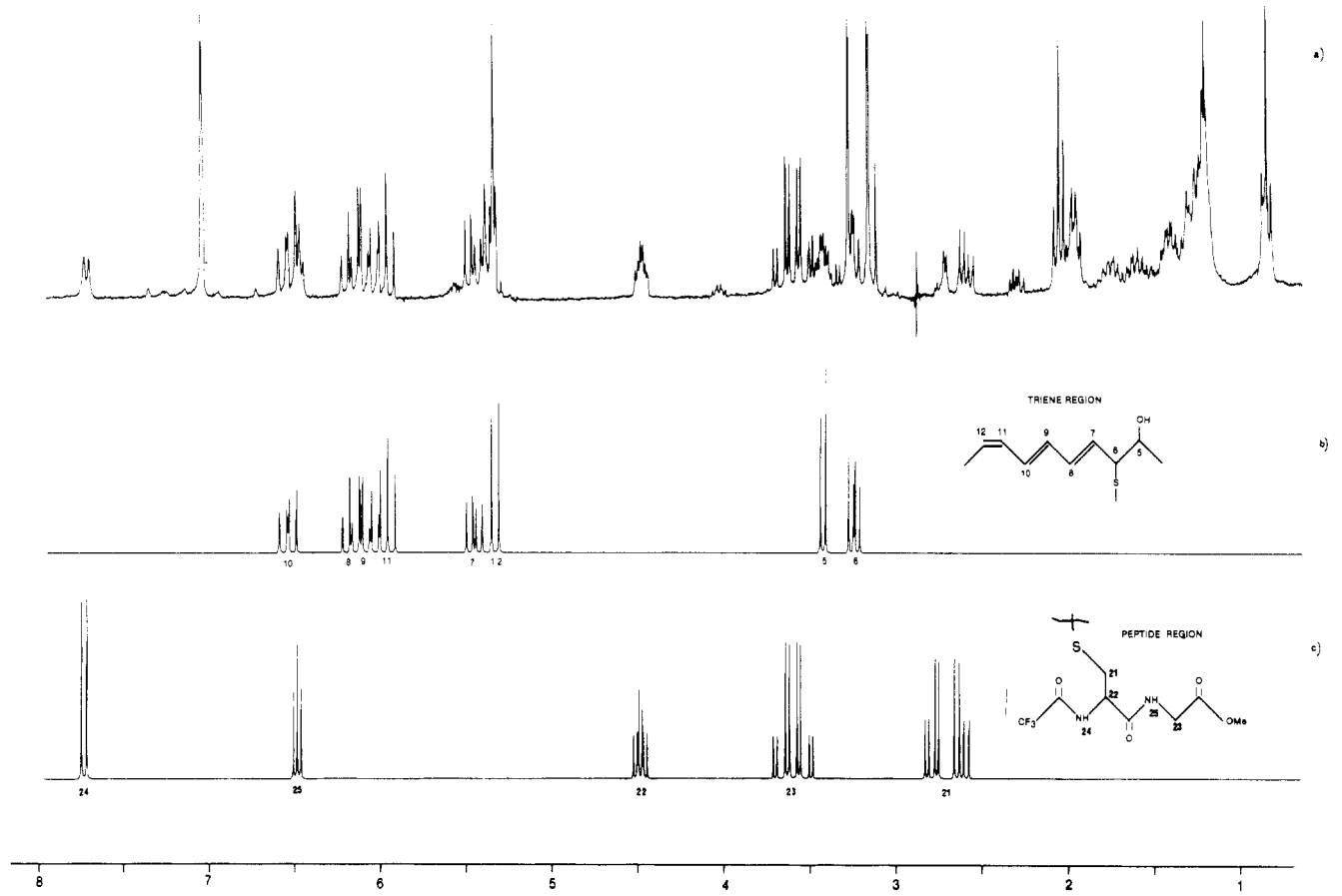


Figure 2. (a) 250-MHz proton spectrum of VIIA in C_6D_6 solution with homonuclear decoupling at δ 2.96 to remove the coupling to the diallylic methylene protons; (b) partial simulated spectrum of the olefinic region of VIIA; (c) partial simulated spectrum of the amino acid resonances of VIIA.

Table II. Proton Chemical Shifts and Coupling Constants for LTD Analogues

	chemical shift, δ							coupling constant, Hz			
group	VIIA[a]	VIIB[a]	VIIC[a]	VIID[a]	VIII[b]	IX[e]	J	VIIA[a]	VIIB[a]	VIIC[a]	VIID[a]
2	2.14	2.14	2.09	2.11	2.26	2.24	2,3	7.1	6.9		7.2
3	1.50	1.46	1.48	1.44	1.5-1.7		5,6	7.3[c]	7.6	1.9	1.9
4	1.68	1.65	1.69	1.62	1.5-1.7		6,7	9.5[c]		9.5	9.6
5	3.56	3.55	3.65	3.69	3.76	3.62[f]	7,8	14.6[c]	15.0	14.2	14.4
6	3.32[c]	3.54	3.55	3.48	3.42	3.36[f]	8,9	11.1[c]	10.8	11.4	10.5
7	5.56[c]	5.51	5.62	5.74	5.47	5.66	9,10	14.4[c]	15.0	14.2	14.3
8	6.28[c]	6.49	6.52	6.35	6.30	6.27	10,11	11.2[c]	11.5	11.4	11.5
9	6.18[c]	6.18	6.16	6.20	6.24	6.21	11,12	11.0[c]	10.8	10.9	11.1
10	6.65[a]	6.73	6.72	6.67	6.60	6.57	12,13	5.3[d]	6.4[d]		6.2[d]
11	6.07[c]	6.08	6.07	6.09	6.03	6.02	13,14	5.3[d]	6.4[d]		6.2[d]
12	5.43[c]			5.4-5.5			15,16	6.4	6.6		
13	2.96	2.97	2.94	2.96	2.97	2.95	16,17	6.9	6.6		
14, 15			5.3-5.5				19,20	6.7	6.8		
16	2.03	2.04	2.04	2.03	2.09	2.09	21,21	14.4[c]	14.3	15.2	13.9
17-19			1.2-1.4				21,22	5.4, 7.7[c]	5.3, 8.5	3.3, 9.5	5.7, 7.6
20	0.89	0.90	0.88	0.89	0.90	0.90	22,24	7.5[c]	7.6		
21	2.67[c]	2.80	2.54	2.71	2.63		23,23	18.0[c]	18.0		17.7
	2.84[c]	2.94	2.80	2.79	2.90		23,25	5.3[c]-5.7[c]	5.3-5.7		5.3-5.7
22	4.56[c]	4.72	4.46	4.52							
23	3.72[c]	3.91	3.73	3.73	3.66	3.63					
	3.60[c]	3.79	3.69	3.58	3.93	3.79					
24	7.85[c]	8.20	7.86	7.76							
25	6.59[c]	7.34		6.60							
26	3.50										
27	3.35	3.35	3.31	3.34							
28	3.26	3.28	3.20								

[a] Sample consists of ca. 15 mg of compound in 1.0 mL of C_6D_6. [b] Sample consists of ca. 3 mg of compound in 1.0 mL of CD_3OD. [c] Iterated second-order value (rms deviation 0.13 Hz). [d] Averaged value. [e] Sample consists of ca. 1 mg of compound in 0.5 mL of CD_3OD. [f] Tentative assignments.

of complexity, the spectrum is generally well resolved with many immediately recognizable groups. In this case, however, selective decoupling of the diallylic protons at 13 leaves a residual olefinic fragment which is still too large for iterative simulation. Consequently, only the protons between 12 and 5 have been considered. The effect of this

limitation can clearly be seen in Figure 2 where the methine proton (5) appears as a simple doublet in the theoretical spectrum as opposed to the distinctive doublet of triplets observed experimentally as a result of the additional coupling to the methylene protons at 4. Also of note in this context is the complex appearance of the experimental spectrum around δ 5.5 due to the signals from the two additional protons at 14 and 15 which have also been omitted from the theoretical study and the two well-separated sets of signals for the cysteine methine proton (22) at δ 4.56 and 4.1. These are believed to arise from the presence of two separate hydrogen-bonded species in solution as the effect disappears on changing the solvent or sample concentration.

The ABX and ABMX spectra formed by 23 and 25 and by 21, 22, and 24, respectively, have also been modeled against the undecoupled spectrum, and these results are also shown in Figure 2. In all cases, the root-mean-square error in the fit was better than 0.13 Hz, and the complete set of results obtained are given in Table II. Also included in this table are the results for the deprotected 5(*S*),6(*R*) (IX) and 5(*R*),6(*R*) (VIII) diastereomers which, to the best of our knowledge, constitute the first reported data for leukotriene D_4 in the deprotected form.

The chemical shifts for VIIC and VIID show good general agreement with those reported by Rackham et al.[11] in deuteriochloroform solution. Once again, a high-field shift appears to occur in benzene-d_6 with the values of the aliphatic ester methyl (27) in VIIC and VIID changing from δ 3.36 and 3.66 in deuteriochloroform to δ 3.31 and 3.34 in benzene-d_6.

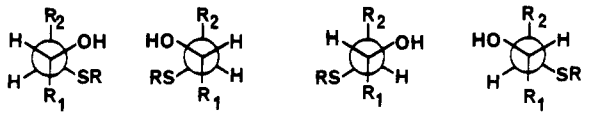
Discussion

The magnitude of the appropriate vicinal proton coupling constants clearly substantiates the geometry about the olefinic double bonds for all the compounds studied with double bonds in the cis configuration, giving a coupling of 11–11.5 Hz, while the values for the corresponding trans configuration lie between 14 and 15 Hz.

The leukotriene D_4 analogues can be considered as being made up of three component parts, an unsaturated hydrocarbon chain, a short saturated aliphatic ester tail, and an amino acid side chain, each of which is linked at C_6. Indeed, the importance of the configuration of the C_5 and C_6 centers is clearly demonstrated by the marked differences in biological potency for the four isomers of which VIID is by far the most active.[9]

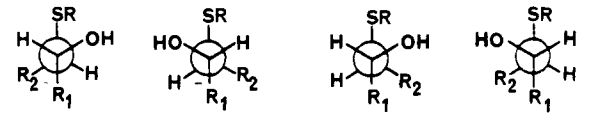
For effective conjugation to be maintained, C_7, C_8, C_9, C_{10}, C_{11}, and C_{12} are constrained to lie in the same plane as the methine proton at 7. The magnitude of the vicinal coupling between the protons at 6 and 7 in VIIA, VIIC, and VIID is 9.5, 9.5, and 9.6 Hz, respectively, indicating that all three compounds have a similar conformational preference about this bond. The value of ca. 9.5 Hz shows that these two protons exist in a predominantly trans conformation in all three compounds. The magnitude of the corresponding 5–6 coupling is 7.3 Hz in VIIA, 7.6 Hz in VIIB, 1.9 Hz in VIIC, and 1.9 Hz in VIID where the two small values are clearly indicative of a gauche coupling. The two most likely conformations for each of these isomers are those in which the aliphatic ester side chain is *trans* to either the olefinic or amino acid side chain.

The conformations with the aliphatic ester chain trans to the olefinic chain are



VIIA VIIB VIIC VIID

and the conformations with the aliphatic ester chain trans to the amino acid chain are



VIIA VIIB VIIC VIID

For the first of these possibilities, VIIA and VIIB would each give rise to a gauche $^3J_{5,6}$ coupling while VIIC and VIID would give the corresponding trans $^3J_{5,6}$ coupling so that the value in the last two compounds would be expected to be greater than that in the first two. This is clearly not the case. If, however, the aliphatic ester chain is trans to the amino acid chain, then the positions are reversed, and the first two compounds now correspond to a trans coupling while the latter two compounds correspond to a gauche coupling. This situation is in good overall agreement with the experimentally observed results, but it is clear that both VIIA and VIIB each contain a significant proportion of the appropriate gauche conformation since the observed values of 7.3 and 7.6 Hz are too small to be pure trans coupling constants.

Due to the absence of any appropriate vicinal proton coupling, the relative orientation of the amino acid side chain is undefined, and the magnitudes of the two vicinal cysteine side chain couplings of 5.4 and 7.7 Hz are such that there appears to be more than one significantly populated conformation about this bond. A similar consideration applied to the other coupling values along the amino acid side chain. Consequently, it appears that this portion of the molecule exhibits relatively little conformational preference and that there are several well-populated forms.

Compounds VIIC and VIID were also studied in the deprotected form (VIII and IX) in methanol-d_4 solution. Under these conditions, the spectra were generally less well resolved, and only the chemical shift values for these two compounds are recorded in Table II. The values are generally similar to those obtained for the protected compounds and imply an overall similarity in the conformational profile of the two molecules. This conclusion is supported by the values of the 5,6 and 6,7 vicinal couplings in VIII which, at 2.1 and 10.5 Hz, are similar to the corresponding values in the protected compound of 1.9 and 9.5 Hz, respectively. The corresponding values in IX could not be obtained due to the broad nature of the resonances involved, but it would seem that the conclusions drawn here for the protected compounds may also hold true for their deprotected counterparts.

On the basis of the results obtained, the leukotriene D_4 analogues appear to be characterized by a rigid olefinic backbone and a marked preference for a trans orientation between the methine protons at 6 and 7, producing a clearly defined orientation of the cysteine sulfur atom relative to the olefinic side chain. An additional, but less strongly defined, preference for a trans orientation exists between 5 and 6 for the aliphatic ester group and the amino acid side chain. The remainder of the molecule

(11) Rackham, D. M.; Morgan, S. E.; Paschal, J. W.; Elzey, T. K. *Spectrosc. Lett.* **1981**, *14*, 589.

appears to have a considerable degree of conformational freedom with significant populations of more than one rotamer about each bond.

Acknowledgment. We thank Dr. A. K. Willard and Mr. J. J. Maloney for helpful discussions and experimental assistance.

Registry No. II, 75415-32-6; III, 83831-83-8; IV, 72297-11-1; V, 72345-92-7; VI, 72297-14-4; VIIA, 83861-12-5; VIIB, 83861-13-6; VIIC, 80559-09-7; VIID, 76322-44-6; VIII, 81026-46-2; IX, 73836-78-9.

Synthesis and Photooxidation of the Condensation Products of Tryptamine and Catechol Derivatives. An Approach to the Synthesis of a Probable Precursor of Koumine

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Two tryptamine–catechol derivative condensation products, 1,3,5,6,14,21-hexahydro-17,18-dihydroxybenz-[*g*]indolo[2,3-*a*]quinolizine (**2**) and 3-ethoxy-3,4-seco-*N*-methyl-1,3,5,6,14,21-hexahydro-17,18-dihydroxybenz-[*g*]indolo[2,3-*a*]quinolizine (**5**), as intermediates for the probable precursor **11** of the *Gelsemium* alkaloid koumine have been synthesized, and the catechol rings of these compounds were successfully cleaved by photosensitized oxidation in which the two muconic acid derivatives **7** and **8** have been obtained. Compound **8** may be regarded as a precursor of **11**.

Koumine, the principal alkaloid of *Gelsemium elegans* Benth, was first isolated by Chao in 1931,[1] and its complete structure has been established by our group recently.[2]

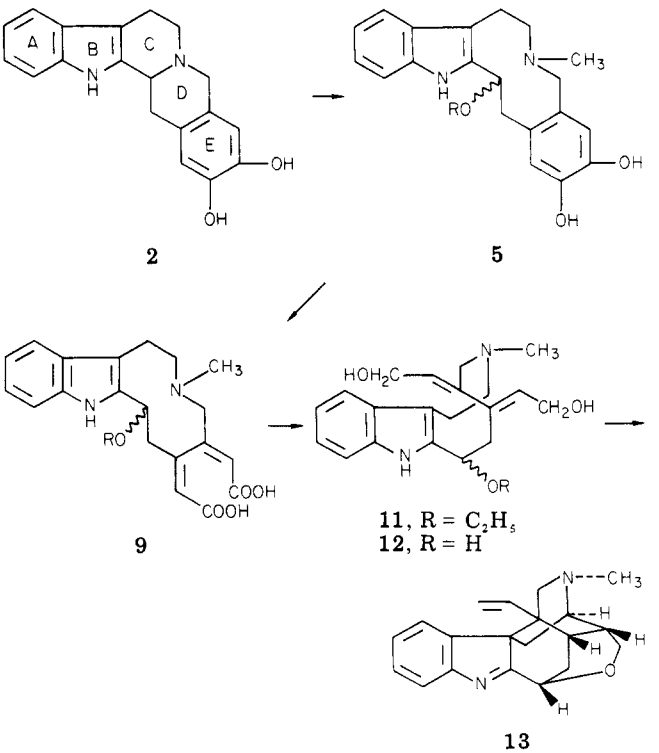
The first stage of our approach to the synthesis of koumine **13** is based on Woodward's biogenetic hypothesis[3] of strychnine. Although this hypothesis is not consistent with the current biogenetic theory of indole alkaloids,[4] the total synthesis of strychnine,[5] which was designed according to this biogenetic hypothesis, has been accomplished successfully. It is interesting that the cleavage of the catechol ring by oxidation affords a product similar in structure to the tryptamine secologanine condensation derivatives (cf. **5**–**12**, Scheme I).

The first stage of our approach is the synthesis of compound **2** and the opening of the C–D ring fusion to give **5**. Photooxidation of **5** would provide the fission products **9**, which could eventually lead to **12**, a possible key precursor of koumine.

Compound **2** (Scheme II) was synthesized from tryptamine and 3,4-dihydroxyphenylpyruvic acid, according to the procedure of Harley-Mason.[6] The substituted pyruvic acid in turn was synthesized from vanillin and acetylglycine.[7,8]

Harley-Mason believed that the condensation in the Mannich reaction (**1** to **2**) took place between N_4 and C_{20}. This was based on comparison of compound **2** with some relevant known structures. However, there had been an earlier report that the site of condensation, i.e., the cyclization of rings C and E, might be between N_4 and C_{16}.[9] We are now able to show that the condensation has unambiguously taken place between N_4 and C_{20} instead of

Scheme I



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