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MUCRONATININE, A NEW ALKALOID FROM *CROTALARIA MUCRONATA* DESV.—I

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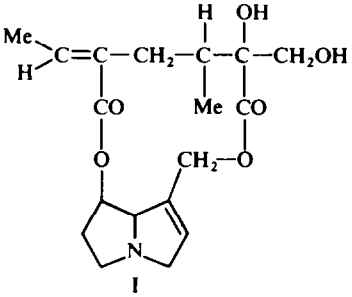
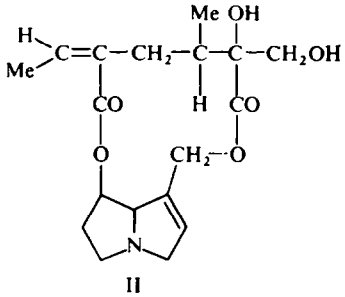
Abstract—A new alkaloid mucronatinine, has been isolated from *Crotalaria mucronata*. Its structure has been established using NMR and mass spectra. It has been found to be a diastereomer of retrorsine. The antimicrobial screening of mucronatinine has also been reported.

Crotalaria mucronata Desv., a shrub which grows in Assam, India, is widely used in the southern part of the U.S.A. as a green manure crop under the name "giant striata".[1] The genus Crotalaria contains pyrrolizidine alkaloids. Recently, these alkaloids have assumed significant importance due to their mutagenic[2] and anti-cancer properties.[3]

The crude alkaloidal isolate obtained from the seeds of this species by TLC[4] showed the presence of one major (R_f 0·29) and two minor alkaloids. We report here the isolation and structure of the major alkaloid mucronatinine, $C_{18}H_{25}NO_6$, m.p. 161–163°, which is a diastereomer of retrorsine.[5]



I



II

The IR spectrum of mucronatinine (I) in KBr showed saturated (1735 cm^{-1}) and unsaturated (1720 cm^{-1}) ester peaks. Comparison of 220 MHz† NMR spectrum of the known compound retrorsine (II) with that of mucronatine and the identical proton count (25 hydrogens) in the two compounds by the electronic integration of the

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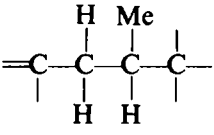
† The NMR spectral data were obtained by running 5 to 6% solns of the two alkaloids on Varian HR-220 super conductive NMR spectrometer.

various signals occurring in their NMR spectra show that the two compounds are diastereoisomers. The essential differences between the NMR spectra of the two alkaloids lie in the resonances of the protons occurring in the macrocyclic 12-membered diester ring, since the NMR signals caused by the pyrrolizidine ring protons are virtually identical.[6a,b]

The resonances for the olefinic 7′-Me and the adjacent vinyl hydrogen in the retrorsine spectrum appear at $\delta = 1{\cdot}86$ and 5·74 respectively, whereas in the mucronatinine NMR trace they occur at $\delta = 1{\cdot}71$ and 6·50 respectively.[6b] The downfield shift of 0·76 ppm for the olefinic proton in the latter spectrum is attributable to the fact that the chemical shift of a vinyl proton *cis* to a CO group appears at a much lower field[7] than that of olefinic hydrogen which is *trans* to the CO function. To a smaller extent, a similar effect may also be observed in the case of olefinic Me proton signals.[8] Therefore it is not surprising to find the vinyl Me resonance in retrosine occurring at a lower field than in the mucronatinine trace. In the two alkaloids spectra not only are the chemical shifts of the olefinic Me hydrogens and the vinyl proton dissimilar, but their NMR splitting patterns are also different. In the mucronatinine trace the signals caused by the olefinic 7′-Me and the vinyl hydrogen appear as slightly broadened doublet and quartet respectively, while in the retrorsine spectrum the resonance for the respective protons are further split by 1·5 Hz due to the long range allylic coupling of these protons to one of the C-4′ methylene group protons. This arrangement was deduced by performing frequency swept double resonance experiments at 100 MHz and will be mentioned in greater detail while examining the various differences in the resonances of the protons occurring in the



part of the two alkaloids.

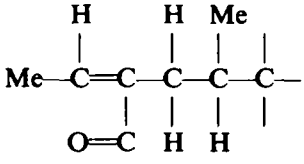
The signals due to the secondary Me protons in retrorsine and mucronatinine appear at $\delta = 0{\cdot}87$ and 0·84 respectively and exhibit spin coupling of 6·00 and 6·75 Hz in the two respective compounds. In the retrorsine spectrum at both 100 and 220 MHz the secondary Me group proton doublet at $\delta = 0{\cdot}87$ is badly perturbed[9] (i.e. the high field side peak is considerably smaller as well as narrower than the low field signal) and also shows considerable amount of filling in the middle[10] which indicates that the resonances of C-3′ hydrogen and at least one of the two C-4′ methylene group protons have very similar chemical shifts. This was confirmed by frequency swept double resonance experiments at 100 MHz. In the first experiment when the Me doublet at $\delta = 0{\cdot}87$ was irradiated, the multiplet at 1·73 ppm became somewhat sharper, thereby locating the chemical shift of the C-3′ methine resonance. During the second double resonance experiment when the multiplet at 2·21 ppm was irradiated, three significant changes took place in the retrorsine spectrum: the disappearance of 1·5 Hz splitting from the olefinic Me doublet at $\delta = 1{\cdot}86$ as well as the vinyl hydrogen quartet at $\delta = 5{\cdot}74$ and the drastic change in the resonance near 1·73 ppm. This means that the proton whose resonance occurs near 2·21 ppm must be coupled to the vinyl 7′-Me and the olefinic hydrogen with an allylic coupling of 1·5 Hz and also coupled to the two nonequivalent protons whose absorption occur near 1·73 ppm. Such a proton must be one of the two hydrogens at C-4′. The

resonance of the other proton belonging to the C-4′ methylene group is very close to that of the C-3′ methine hydrogen.

With the above information from the double resonance experiments at 100 MHz it is now possible to make definitive assignment of the resonances around 2·21 and 1·73 ppm in the 220 MHz spectrum of retrorsine. The apparent triplet near 2·21 ppm is made up of resonances of two hydrogens, namely one of the pyrrolizidine ring protons (most likely H-5u) and one of the two hydrogens at C-4′. It is quite likely that two down field peaks (making up a broad doublet) in this apparent triplet belong to one of the C-4′ hydrogens. The broad doublet at $\delta = 2{\cdot}21$ has a large splitting of 11 Hz which fits rather nicely in the resonance pattern at 1·73 ppm, the latter representing the C-3′ methine proton and the other C-4′ hydrogen. This means that the two non-equivalent protons at C-4′ are spin coupled to each other with a coupling of 11 Hz. Furthermore 11 Hz splitting is found in the entire resonance pattern at 1·73 and it is therefore assumed that C-3′ methine proton and the high field C-4′ proton are also coupled to each other with a coupling 11 c/s. The smaller splitting of 6 c/s observed in the absorption pattern at 1·73 ppm comes from the coupling of C-3′ methine proton to the secondary Me protons. Since the resonance at $\delta = 2{\cdot}21$ representing the low field C-4′ hydrogen is only a broad doublet, it is assumed that the spin coupling between this proton and the C-3′ methine hydrogen is less than or around 1 Hz. The other broadening of the C-4′ hydrogen doublet may be certainly attributed to allylic coupling with the olefinic 7-Me and the vinyl hydrogen.

The assignments of C-3′ and C-4′ hydrogen resonances in the 220 MHz spectrum of mucronatinine were made by comparing them with the NMR data of the respective protons in the retrorsine spectrum. The C-4′ methylene group proton signals in mucronatine spectrum like that of retrorsine show that these hydrogens are nonequivalent. However unlike the retrorsine trace, the resonances caused by these protons are separated from the C-3′ methine proton absorption, which occurs at $\delta = 1{\cdot}735$ and is partially hidden by the two tall signals attributed to olefinic 7′-Me protons. The two nonequivalent C-4′ hydrogen resonances appear at $\delta = 1{\cdot}99$ and 2·16. The broad doublet at $\delta = 1{\cdot}99$ shows a large geminal couling of 13 Hz. The broadening is attributed to the long range allylic coupling with the olefinic 7′-Me protons, the vinyl hydrogen and the adjacent C-3′ methine proton. The rather broadened NMR pattern at $\delta = 2{\cdot}16$ exhibits a large geminal coupling of 13 Hz and almost equally large vicinal coupling of 10·5 Hz to the C-3′ methine hydrogen. The broadening of the resonance is attributed to the long range allylic coupling with the olefinic 7′-Me hydrogens and the olefinic protons. Thus in mucronatinine spectrum both C-4′ hydrogens are spin coupled (most likely unequally) to the olefinic 7′-Me protons and vinyl hydrogen, and hence splitting is not observed in the resonances of the latter protons.

The above examination of the proton resonance dealing with the



skeleton of the two alkaloids clearly shows that not only the molecular arrangement

at C-6′ is dissimilar but the stereochemistry of the secondary Me group at C-3′ relative to the C-3′ methine hydrogen is also different. In one case the Me group is *cis* to the tertiary OH group, while in the other case the relationship is *trans*.

The second assertion was further confirmed upon examination of the two OH group proton resonances in the two compounds. It is well known that when a compound is dissolved in $CDCl_3$ at 5 to 7% concentration and its NMR spectrum is obtained at ambient temperature, the OH proton (located on a saturated C atom) resonates in the range of $\delta = 1{\cdot}5$ to 2·5, whereas the signal for a OH proton which is strongly intramolecularly hydrogen bonded (6-membered ring) appears in the region of $\delta = 5{\cdot}50$ to 6·50. It is also well known that the signals for two or more OH protons coalesce and appear as a single resonance if the OH group protons come close enough to establish an exchange too rapid to be detected by an NMR spectrometer. Such an exchange indeed does take place and can be seen in the NMR spectra of mucronatinine and retrorsine. However the resonance positions of the two OH protons in the two compounds are different. In retrorsine spectrum the OH hydrogens resonate at $\delta = 3{\cdot}20$ whereas in mucronatinine trace they occur at $\delta = 4{\cdot}30$. This observation is in harmony with the speculation that if the secondary Me group in retrorsine were located on the same side as the tertiary OH group, the former group could be crowding the tertiary OH proton into such an exchange with the primary OH proton, where the NMR signal of the two exchanging OH protons appears at a higher field than the one in mucronatine where the tertiary OH group is *cis* to the C-3′ methine hydrogen. Such a relationship between the OH and the adjacent Me groups may very well explain the greater solubility of mucronatinine in aqueous solvents than retrorsine. The above assigned structure of mucronatinine is in excellent accord with its mass spectrum.* The precise mass and the fragmentation patterns obtained for the two alkaloids were identical, once more indicating that mucronatinine and retrorsine are stereoisomers. The main fragmentation pathways of the alkaloids show major ions which are consistent with earlier studies on similar macrocyclic diesters.[11] The following tentative structures can be assigned to the major peaks occurring in the mass spectrum of the two compounds:*

Mucronatinine was tested up to 1600 microgram/mil. concentration against *Staphylocci aureus*, *Escherichia coli*, *Bacillus cerus*, *Sachromycetes cerevisiae*, *Candida albicans* and *Asperigillus niger*, using a serial dilution method[12] and was found inactive.

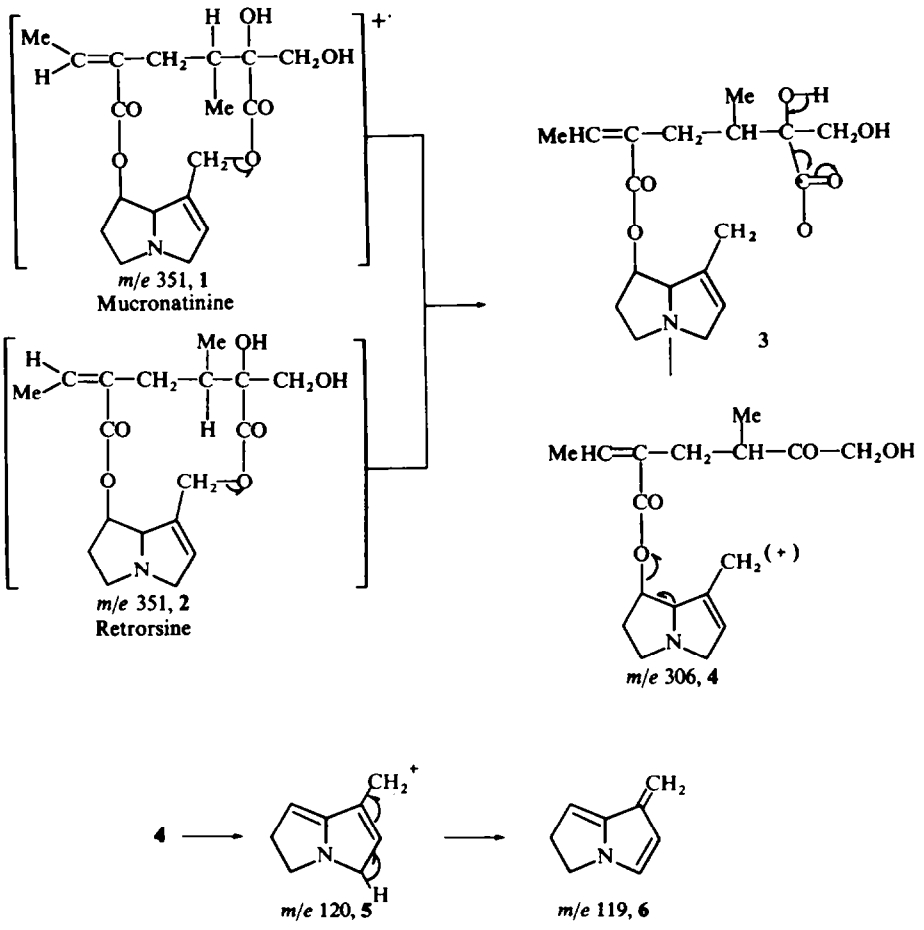
* The mass spectral data were obtained on Varian M-66 using a direct inlet probe.

EXPERIMENTAL

Analyses were done by Galbraith Laboratories, Inc. Knoxville, Tennessee. M.ps were taken on "Thomas Hoover Capillary Melting Point Apparatus" and are corrected.

Isolation of mucronatinine. Milled, dehusked seeds of *Crotalaria mucronata* (1200 G) were defatted with light petroleum (b.p. 40–60°) and extracted with 95% EtOH in a Soxhlet extractor. After removal of the solvent *in vacuo*, the dark brown product was stirred with 5% H_2SO_4 and the soln reduced overnight with Zn dust. The mixture was filtered and made alkaline with 6% NH_4OH and extracted with $CHCl_3$. The crude base showed the presence of 3 alkaloids, R_f (TLC)[4] 0·09, 0·29, 0·60, out of which R_f 0·29 alkaloid was predominant.

Fragmentation pattern of mucronatinine and retrorsine



A benzene–$CHCl_3$ soln of the crude base was chromatographed over a column of alumina (B.D.H. Column Chromatography grade, 75 G) 0·550 mg of the alkaloid of R_f 0·29 was eluted with benzene–$CHCl_3$ Rechromatography of this alkaloid in a similar manner gave crystalline mucronatinine, m.p. 161–163°, mol wt, 351·14 (mass spectrum) which conforms to the molecular formula $C_{18}H_{25}NO_6$.

Mucronatinine picrate, m.p. 228–229°. Mucronatinine picrate, $C_{18}H_{25}NO_6$, $C_6H_3N_3O_7$. Found: C, 49·16; H, 5·40; N, 9·28. Calc.: C, 49·66; H, 4·86; N, 9·64%).

Methiodide of mucronatinine melted at 229·5–230·5°.

Due to the modest quantity of the compound, we could not get the analysis of each derivative. From the total of 185 milligrams of isolated pure alkaloid we were able to do only the structural and biological screening.

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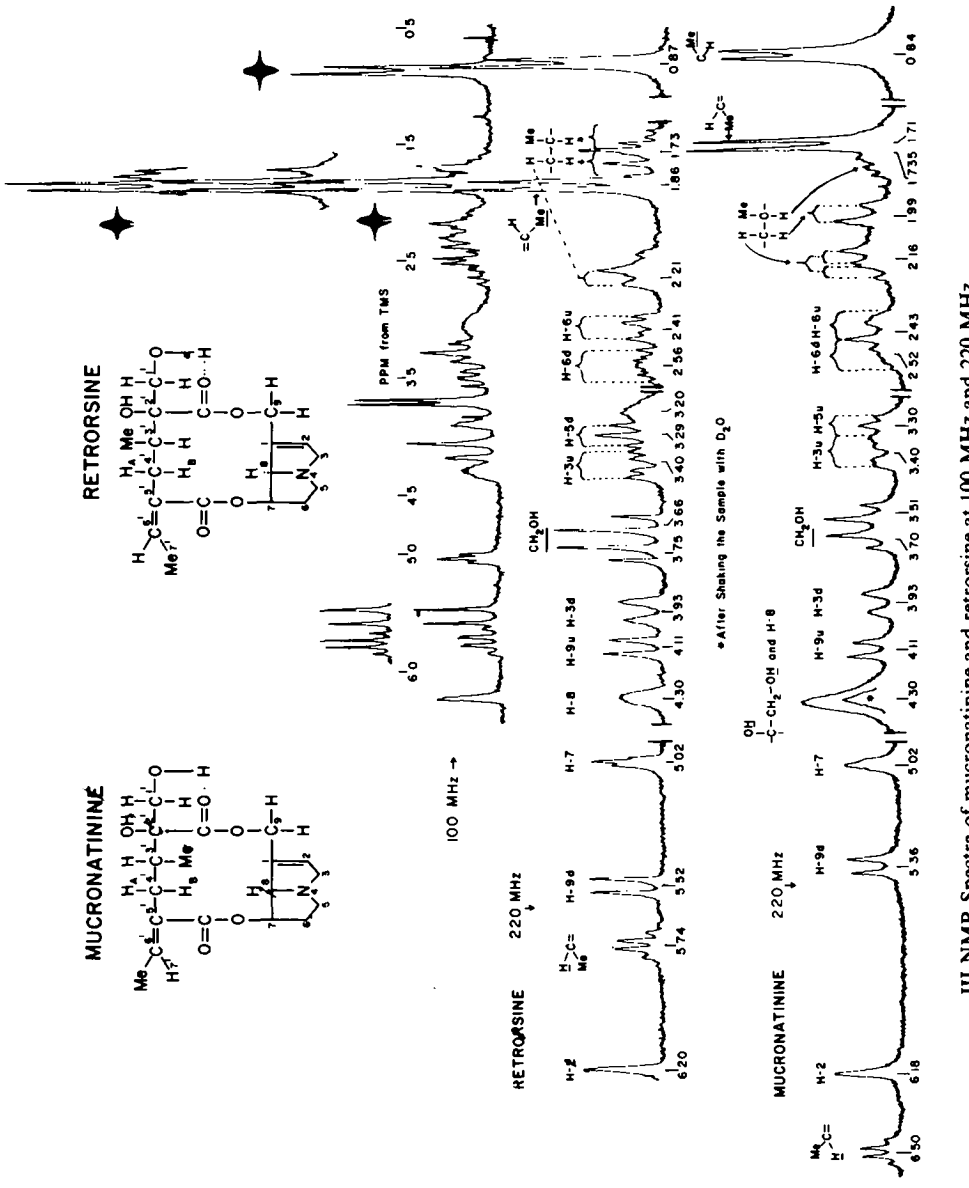
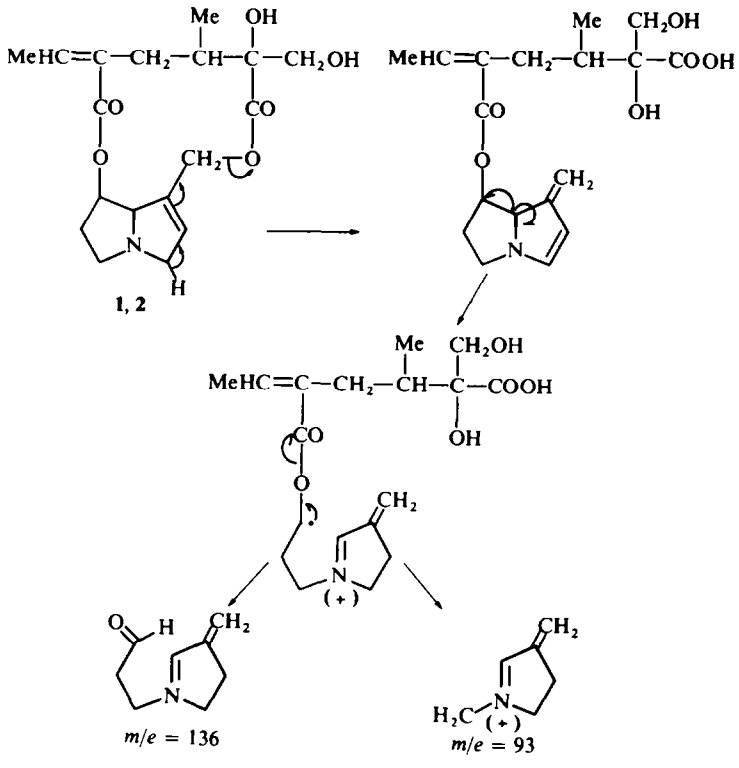
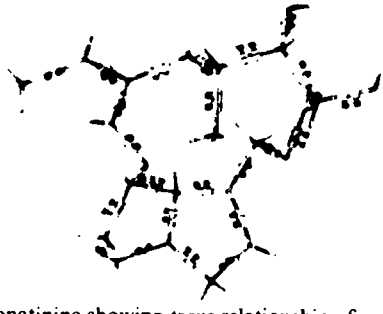


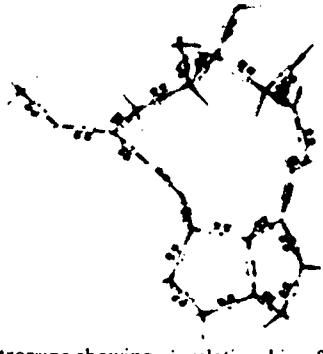
III NMR Spectra of mucronatinine and retrorsine at 100 MHz and 220 MHz.

Formation of *m/e* 136 and 93:





IV Cre model mucronatinine showing *trans* relationship of methyl and hydroxyl groups.



V Cre model of retrorsine showing *cis* relationship of methyl and hydroxyl groups.

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