Tetrahedron Letters No. 14, pp 1233 - 1236. 0040-4039/79/0401-1233$02.00/0

LIGULARIDINE, A NEW PYRROLIZIDINE ALKALOID FROM LIGULARIA DENTATA[1]

Manabu HIKICHI, Yoshihisa ASADA and Tsutomu FURUYA*

School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo, 108 Japan

Summary The structure of a new macrocyclic pyrrolizidine alkaloid, ligularidine(1), having significantly high mutagenic activity was elucidated and the configurations of the necic acid and its enantiomer reported by Edwards[2] were also determined

In the course of the studies on carcinogenic pyrrolizidine alkaloids in Compositae plants, a new macrocyclic pyrrolizidine alkaloid, named ligularidine(1) was isolated from the roots and aerial parts of Ligularia dentata Hara(Japanese name Maruba-dakebuki) together with a known pyrrolizidine alkaloid, clivorine(5)[3]

The present paper deals with the structural determination of ligularidine and its necic acid moiety by chemical and spectral evidences

The crude alkaloids obtained from the MeOH exts of the roots and aerial parts were chromatographed on silica gel column using C_6H_6 AcOEt $HN(Et)_2$ solvent system to afford ligularidine, the mixture of several alkaloids and clivorine which is identical with the authentic specimen by the direct comparisons of mixed m.ps , $[\alpha]_D$ and IR spectrum

Ligularidine(1), colorless needles, m p 196.0° and $[\alpha]_D$ -49 8°(EtOH), shows molecular formula $C_{21}H_{29}O_7N$(M^+ 407 196) and 21 detectable signals of carbons by carbon thirteen nuclear magnetic resonance(CMR) spectrum, of which signals at δ(ppm) 171 9, 169 8 and 167 4 are showing the presence of three ester carbonyl carbons which are also ascertained by IR spectral absorption at ν_{max}^{KBr} cm^{-1} 1740, 1730 and 1700(conj -COOR) The signals at 193 7, 137 5, 134 1, 138 5 and 131 5 show the presence of α,β-unsaturated carbonyl group in the necine moiety and the ethylidene group in the necic acid moiety[4] The proton magnetic resonance(PMR) spectrum of 1 shows the twelve membered macrocyclic seco-pyrrolizidine alkaloid[5] A singlet at δ(ppm) 2 11(3H), a triplet at 4 92(1H) and a broad singlet at 6 16(1H) correspond to CH_3-N at N-4 position, methine proton(-CH-OCO-) at C-7 and an olefinic proton at C-2, respectively The signals of the geminal methylene protons at C-9 position are represented at a pair of doublets at 5 11 and 4 38(J=12 0 Hz each other)[6] From the appreciable difference of the shift (ΔH=0 72ppm) and the coupling constant (J=12 0Hz) between the geminal protons at C-9, 1 is classified as a twelve membered macrocyclic otonecine diester alkaloid These facts are also supported by positive circular dichroism (CD) curves[3,7], at 244nm (Δε +3 91) and 280nm (+0 84). Other assignable signals in the necic acid moiety are at 2 00(3H, s) for acetyl group, 1 45(3H, s) and 0 94(3H, d, J=6 0Hz) for two methyl groups at C-12 and C-13 and 1 78(3H, d, J=8 0Hz) and 6 79(1H, q, J=8 0Hz) for trans-ethylidene group at C-15, respectively

The high resolution mass spectral studies on 1 show that the fragment ions at m/e 168 ($C_9H_{14}O_2N$), 152($C_9H_{14}ON$) and 151($C_9H_{13}ON$) arise from the necine moiety[8] The significant fragment ions at m/e·363($C_{20}H_{29}O_5N$), 320($C_{18}H_{26}O_4N$), 249($C_{14}H_{19}O_3N$), 221($C_{13}H_{19}O_2N$) and 43(100%, C_2H_3O) indicate that the acetoxy group is located at C-12, two methyl groups are attached at C-12 and C-13 positions in the necic acid moiety, respectively (Fig 1)

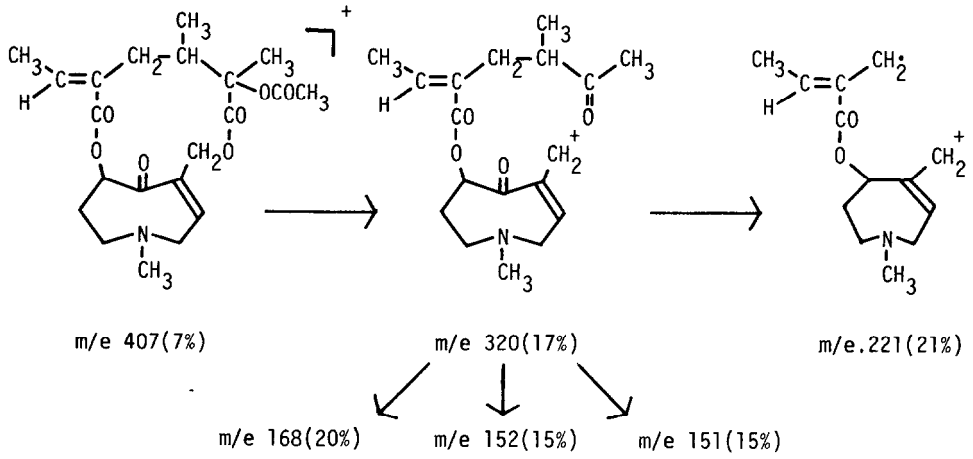

Figure 1

From the data mentioned above, the plane structure of 1 is shown to be 12-acetoxy-trans-15-ethylidene-4,12,13-trimethyl-8-oxo-4,8-seco-senec-1-enine

The absolute configurations of asymmetric carbons in the necine and the necic acid were revealed by chemical hydrogenolysis and hydrolysis Hydrogenetion of 1 with Adam's catalyst and successive hydrolysis gave (7R)-dihydrodesoxyotonecine, b p 135°/10mmHg(picrate, m p 220 0°)[8] On the other hand, alkaline hydrolysis of 1 afforded necine and necic acid which were treated with HCl to yield otonecine hydrochloride(gum)[9], and necic acid lactone(3), m p 131 0°, $[\alpha]_D$ +10 5°(EtOH) and $C_{10}H_{14}O_4$ by mass spectrum The IR(ν_{max}^{KBr} cm^{-1} 3500, 1750 and 1660), PMR δ(ppm) 1 18(3H, d, J=6 0Hz), 1 65(3H, s), 1 80(3H, d, J=8 0Hz), 7 16(1H, q, J=8 0Hz) and 2 0- 2 5(3H, complicated peaks) and the mass fragment patterns of 3 indicate that 3 is 5-carboxy-trans-2-ethylidene-4,5-dimethylpentane-5-olide and furthermore these physical data are in good agreememt with physical constants of (4R,5S)- or (4S,5R)-5-carboxy-trans-2-ethylidene-4,5-dimethylpentane-5-olide reported by J D Edwards[2] The positive CD curves of 3(Δε+2 79 at 225nm and +0 26 at 262nm) and its dihydronecic acid lactone (4)(Δε +3 99 at 225nm) indicate that an absolute configuration at C-5 in 3 is (S)[10] Therefore 3 is suggested to be (4R,5S)-5-carboxy-trans-2-ethylidene-4,5-dimethylpentane-5-olide

A further study was carried out by the direct preparation of 1 from clivorine (5) Partial hydrogenation with Raney nickel catalyst yielded 1 which is identical with naturally occurring ligularidine by direct comparison of mixed m ps , $[\alpha]_D$ and other spectral data (Fig 2)

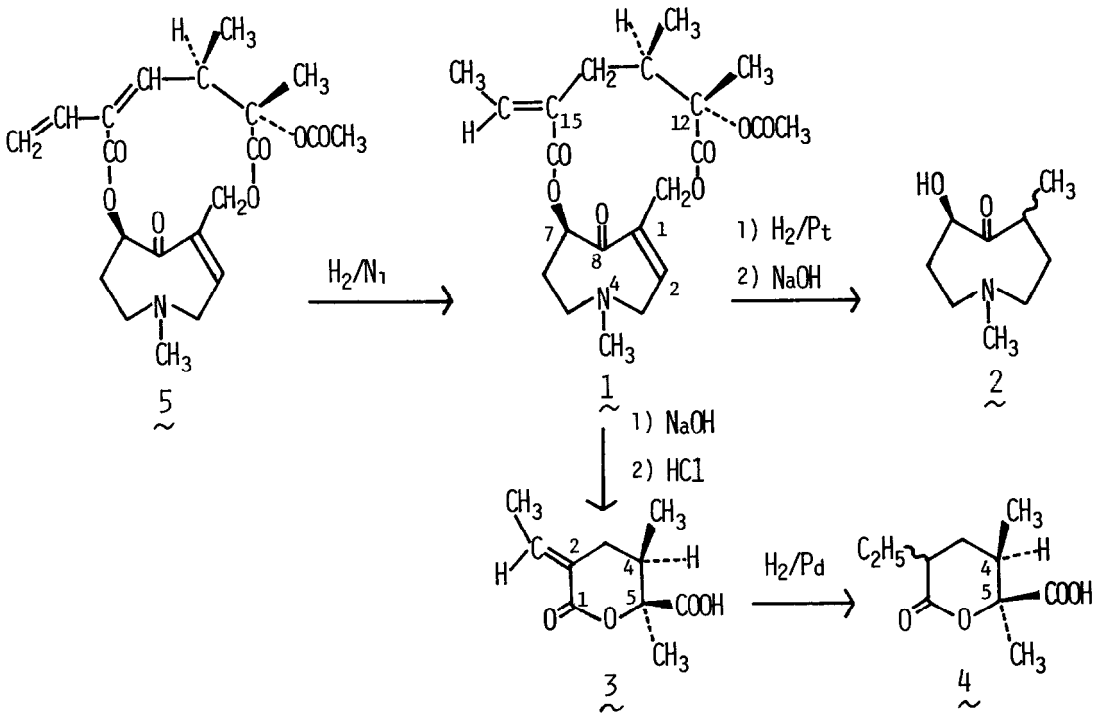

Figure 2

From the spectral and the chemical data mentioned above, the structure of ligularidine(1) was elucidated to be (12S,13R)-12-acetoxy-trans-15-ethylidene-4,12,13-trimethyl-8-oxo-4,8-seco-senec-1-enine and (+)-acid and (-)-acid lactones reported by Edwards[2] were also concluded to be (4R,5S)-acid lactone(3) and (4S,5R)-acid lactone[11], respectively

The necic acid moiety in ligularidine(1) shows the characteristic structure having trans-ethylidene group at C-15 and unusual (S)-configuration at C-12 among many macrocyclic secopyrrolizidine alkaloids except clivorine(5)[3] Now it became just urgent to test the carcinogenic activities like as fukinotoxin[12], because 1 and its significant pyrrolic derivative[13] showed high mutagenic activities[14] and more the tribe Senecioneae plants are widely used as foods and medicinal crude drugs[15] in Japan.

The tests of carcinogenicity and other biological activities are now in progress.

Acknowledgements —— The authors wish to thank to Professor F Šantavý, Chemical Institute Medical Faculty, Palacký University, for a gift of the authentic sample of clivorine and this work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan

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*All melting points are uncorrected and the molecular formulae were measured by high resolution mass spectrometer and the analytical values were in good agreement with the calculated values.

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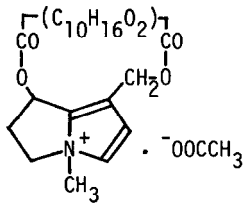

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(Received in Japan 25 December 1978)