HETEROCYCLIC NITROGEN-CONTAINING COMPOUNDS FROM THE EXTRACT OF THE SPRINGTAIL *Tetrodontophora bielanensis* (WAGA)

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Two pyrido[2,3-*b*]pyrazines were isolated from the extract of the springtail *Tetrodontophora bielanensis* (WAGA). Their structures were determined by mass and NMR spectroscopy: 2,3-dimethoxypyrido[2,3-*b*]pyrazine (*I*) and 2-methoxy-3-isopropylpyrido[2,3-*b*]pyrazine (*II*).

In our preceding paper[1] we described the analysis of the lipidic substances from the extract of the springtail *Tetrodontophora bielanensis* (WAGA) (Insecta: *Collembola*). From the last chromatographic fractions two polar compounds were isolated in pure state. The subject of this paper is the determination of their structures.

EXPERIMENTAL

The isolation of compounds *I* and *II* and the chromatographic methods used are described in our preceding paper[1]. Compound *I* formed white needlelike crystals with m.p. 125–125·5°C (determined on a Kofler block, uncorrected). Compound *II* was not obtained in crystalline state.

Hydrogenation in Acetic Acid[2]

2,3-Dimethoxypyrido[2,3-*b*]pyrazine (*I*) or 2-methoxy-3-isopropylpyrido[2,3-*b*]pyrazine (*II*) (10 mg) was dissolved in glacial acetic acid (2·5 ml, stabilized with CrO_3 and crystallized) and hydrogenated in the presence of a palladium catalyst (20 mg, 5% Pd on charcoal) at room temperature and atmospheric pressure for 20 h. The mixture was filtered, freed of acetic acid and purified by thin layer chromatography on silica gel (5 × 20 cm, light petroleum–diethyl ether 1 : 1). 2,3-Dimethoxy-5,6,7,8-tetrahydropyrido[2,3-*b*]pyrazine (*IV*) (5·2 mg) or 2-methoxy-3-isopropyl-3,4-dihydropyrido[2,3-*b*]pyrazine (*III*) (4·3 mg) were thus obtained.

Hydrogenation in Ethanol[2]

2,3-Dimethoxypyrido[2,3-*b*]pyrazine (*I*) (7·6 mg) was dissolved in ethanol (2 ml) and hydrogenated on palladium (15·8 mg, 5% Pd/C) at room temperature and atmospheric pressure for 8 h. The mixture was filtered, ethanol evaporated and the residue purified as described above. The products obtained were 2,3-dimethoxy-5-ethyl-5,6,7,8-tetrahydropyrido[2,3-*b*]pyrazine (*V*) (1·1 mg) and 2,3-dimethoxy-5,6,7,8-tetrahydropyrido[2,3-*b*]pyrazine (*IV*) (2·5 mg).

Attempt at Hydrogenation in Trifluoroacetic Acid[3]

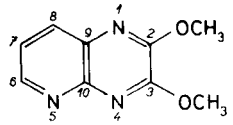
In this attempt, compound *I* was stirred in trifluoroacetic acid in the presence of 5% Pt/C or 5% Pd/C and under hydrogen, but no consumption of hydrogen was observed and only the starting material was isolated from the reaction mixture. When PtO_2 (refs[4,5]) in acetic acid under addition of perchloric acid[6] was used, a destruction of compound *I* always took place (determined by TLC and GLC).

Reduction with Lithium Aluminium Hydride[5]

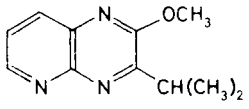
Compound *I* (4·2 mg, 0·022 mmol) or *II* (5·5 mg, 0·027 mmol) was dissolved in 2 ml of diethyl ether and reduced, under stirring, with an ethereal solution of $LiAlH_4$, containing 1·9 mg (0·05 mmol) or 2·0 mg (0·052 mmol) of $LiAlH_4$, respectively, for 30 min at room temperature. The mixture was decomposed[7], extracted with four 5 ml portions of diethyl ether and the extract dried over $MgSO_4$. After purification by TLC on silica gel (5 × 20 cm, diethyl ether) 2-methoxy--3,4-dihydropyrido[2,3-*b*]pyrazine (*VI*) (1·6 mg) was obtained from compound *I*, and 2-methoxy--3-isopropyl-3,4-dihydropyrido[2,3-*b*]pyrazine (*III*) (3·7 mg) from compound *II*.

Mass spectra were measured on AEI MS 902 (Associated Electric Industries, Manchester, G.B.) with double focussing. The samples were introduced into the electron impact ion source *via* direct inlet. The spectra were recorded at about 100°C in the ion chamber, at electron energy of 70 eV and the resolving power about 1 000. Accurate masses were measured at 10 000 resolving power. Partial mass spectra of compounds *I*—*V* are given in Table I.

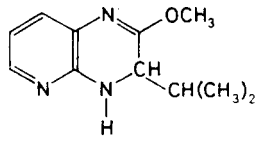
NMR *spectra* were measured on a FT-NMR spectrometer Varian XL-200 (^1H at 200 MHz; ^{13}C at 50·31 MHz) in deuteriochloroform (Aldrich, 99·8% ^2H), using tetramethylsilane as internal standard, at about 22°C. The ^1H NMR spectrum of compound *III* and the mixture of compounds *VI* and *VII* was also measured in hexadeuteriodimethyl sulfoxide (Aldrich, 99·9% ^2H). The ^{13}C NMR spectra (proton decoupled) were measured only with compound *I* and *II*. The type of the carbon atom (C, CH, CH_2, CH_3) corresponding to individual signals was determined from the "attached proton test" spectra[8], intensities of the signals and the chemical shift arguments. The NMR parameters of compounds *I*—*IV*, *VI* and *VII* are given in Table II.



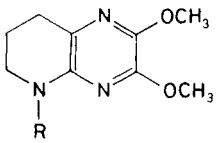
I



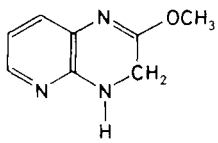
II



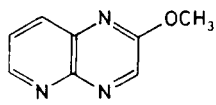
III



IV, R = H
V, R = CH_2CH_3



VI



VII

RESULTS AND DISCUSSION

The compositions $C_9H_9N_3O_2$ for *I* and $C_{11}H_{13}N_3O$ for *II* were determined from the accurate measurement of masses; the number of carbon and hydrogen atoms in both compounds was confirmed by ^1H and ^{13}C NMR spectra (Table II). The ^1H NMR spectra proved the presence of three vicinal aromatic hydrogen atoms in both compounds, the remaining hydrogen atoms belong to the two methoxy groups in *I* and the methoxyl and isopropyl groups in *II*. The considerable downfield shift of one of the aromatic hydrogen atoms (δ 8·78 or 8·89 in *I* or *II*, respectively) and the distinctly decreased value of its ortho coupling (J = 4·4 Hz) indicated the presence of nitrogen atom in vicinal position. These data, together with the 7 aromatic carbon atoms (determined from ^{13}C NMR spectrum) and three nitrogen atoms (from MS) showed that both compounds contain a pyridine ring, annellated in

TABLE I
Partial MS data of compounds *I*—*V*

Compounds	*m/z* (relative abundance, elemental composition[a])
I	38 (3); 39 (3); 40 (3); 51 (2); 52 (3); 53 (6); 54 (3); 64 (7); 65 (3); 76 (3); 77 (3, C_5H_3N); 78 (9); 79 (3); 91 (11); 103 (3); 104 (32); 105 (11, $C_5H_3N_2$); 106 (3); 119 (7, $C_6H_3N_2O$); 120 (4); 130 (4); 131 (18); 132 (13); 133 (7, $C_6H_3N_3O$); 134 (5); 148 (4); 160 (4); 161 (54, $C_7H_3N_3O_2$); 162 (64); 163 (6); 176 (12); 190 (25); M^+ 191 (100, $C_9H_9N_3O_2$); 192 (13).
II	39 (4); 40 (3); 41 (5); 43 (3); 64 (5); 65 (4); 76 (4); 77 (3, C_4HN_2 60% and C_5H_3N 40%); 78 (3); 91 (15, $C_5H_3N_2$); 92 (4); 103 (6); 104 (7); 119 (15, $C_6H_3N_2O$); 120 (5); 145 (4); 146 (6); 158 (3); 160 (10); 162 (4); 170 (3); 172 (3, $C_9H_6N_3O$ 50% and $C_{10}H_{10}N_3$ 50%); 175 (5); 188 (100, $C_{10}H_{10}N_3O$); 189 (13); 202 (6); M^+ 203 (46, $C_{11}H_{13}N_3O$); 204 (7).
III	39 (10); 41 (4); 66 (3); 78 (3); 91 (4); 92 (5); 93 (4); 104 (3); 119 (5); 120 (5); 132 (5); 133 (5); 134 (16, $C_7H_8N_3$); 162 (100, $C_8H_8N_3O$); 163 (17); M^+ 205 (16, $C_{11}H_{15}N_3O$); 206 (3).
IV	41 (6); 68 (8); 84 (7); 97·5 (4); 111 (6); 123 (4); 137 (6); 152 (21, $C_7H_8N_3O$); 165 (6); 180 (79, $C_8H_8N_3O_2$); 181 (10); 194 (9); M^+ 195 (100, $C_9H_{11}N_3O_2$); 196 (14).
V	39 (10); 41 (24); 42 (11); 43 (11); 44 (23); 55 (14); 56 (12); 57 (11); 67 (7); 68 (14); 69 (9); 95 (10); 180 (22, $C_9H_{14}N_3O$); 208 (100, $C_{10}H_{14}N_3O_2$); 209 (15); M^+ 223 (73, $C_{11}H_{17}N_3O_2$); 224 (10).

[a] When determined.

TABLE II

NMR Parameters of compounds *I*–*IV*, *VI* and *VII* in deuteriochloroform

Compound	^{1}H NMR (Chemical shifts and coupling constants)													
	H-3	H-6	H-7	H-8	OCH_3	CH	$(CH_3)_2$	NH	$J_{3,CH}$	$J_{3,NH}$	$J_{6,7}$	$J_{7,8}$	$J_{6,8}$	J_{CH,CH_3}
I	—	8·78 dd	7·48 dd	8·15 dd	4·26 s 4·18 s	—	—	—	—	—	4·4	8·1	1·9	—
II	—	8·89 dd	7·57 dd	8·19 dd	4·14 s	3·55 m	1·41 d	—	—	—	4·4	8·2	1·8	6·8
III	4·19 d	7·67 dd	6·58 dd	7·23 dd	3·90 s	2·11 m	0·89 d 0·99 d	[a]	3·8	[a]	5·3	7·5	1·6	7·0 7·0
III[b]	4·06 dd	7·64 dd	6·48 dd	7·09 ddd	3·80 s	1·89 m	0·79 d 0·86 d	6·95[b]	4·0	2·4	5·1	7·4	1·7	6·8 7·0
IV	—	3·50 t	2·02 m	2·77 t	3·92 s 3·93 s	—	—	[a]	—	—	5·5	6·5	0	—
VI	4·22 s	7·74 dd	6·61 dd	7·22 dd	3·91 s	—	—	4·78[b]	—	[a]	5·2	7·3	1·7	—
VII	8·69 s	8·95 dd	7·63 dd	8·21 dd	4·13 s	—	—	—	—	—	4·3	8·2	2·0	—

	^{13}C-NMR (Chemical shifts)[c]									
	C-2	C-3	C-6	C-7	C-8	C-9	C-10	OCH_3	CH	$(CH_3)_2$
I	150·7 (4·4)	152·8 (4·8)	147·8 (−6·7)	122·2 (−3·5)	136·0 (−2·8)	132·8 (−5·8)	147·0 (−4·6)	54·9 55·3	—	—
II	160·5 (14·2)	156·5 (8·5)	148·1 (−6·4)	124·2 (−1·5)	136·9 (−1·9)	134·7 (−3·9)	147·3 (−4·3)	54·4	31·0	20·3

[a] The value of the parameter could not be determined; [b] data in hexadeuteriodimethyl sulfoxide; $J_{8,NH} = 0·8$ Hz; [c] chemical shifts differences referred to unsubstituted pyrido[2,3-*b*]pyrazine (ref.[7]) are given in parentheses.

position 2, 3 to the diazabenzene cycle carrying both substituents on the carbon atoms. From the six possible types of bicyclic structures A–F (see Fig. 1) three could be excluded by comparison of the mass spectra of our compounds *I* and *II* with the published spectra of similarly substituted compounds of type B (ref.[9]), C (refs[9,10]) and D (ref.[11]). While all the published spectra of the types B ,C and D display significant ions $(M-N_2)^+$ (created by the decomposition of the pyridazine ring), these ions do not occur in the spectra of *I* and *II*. The evidence permitting to ascribe the structural type A to our substances was also obtained from an analysis of their mass spectra. The most convincing argument is the unusual elimination of the fragment C_2H_6 from the molecular ion of compound *I*, affording a prominent ion *m/z* 161 ($C_7H_3N_3O_2$; the observed metastable ion m* 135·7 corresponds to the transition 191 → 161). For this fragmentation (Scheme 1 and 2), the same as for the elimination (C_2H_6 + H) from the molecular ion of compound *II* (Scheme 3) it is necessary that the substituents R and R′ be in vicinal positions. The structural types E and F with a pyrimidine nucleus do not satisfy this requirement and therefore they can be excluded. Hence, the compound *I* may be assigned the structure of 2,3-dimethoxypyrido[2,3-*b*]pyrazine (*I*). The structure *I* is fully consistent with the ^1H and ^{13}C NMR data (Table II). For the assignment of the signals of some carbons in the ^{13}C NMR spectra, data for unsubstituted pyrido[2,3-*b*]pyrazine from ref.[12] were employed, where the assignment was confirmed by the spectra of selectively ^{13}C-enriched derivatives. The substitution with the methoxy groups in positions 2 and 3 in compound *I* causes a downfield shift of atoms $C_{(2)}$ and $C_{(3)}$ by 4·4 or 4·8 ppm

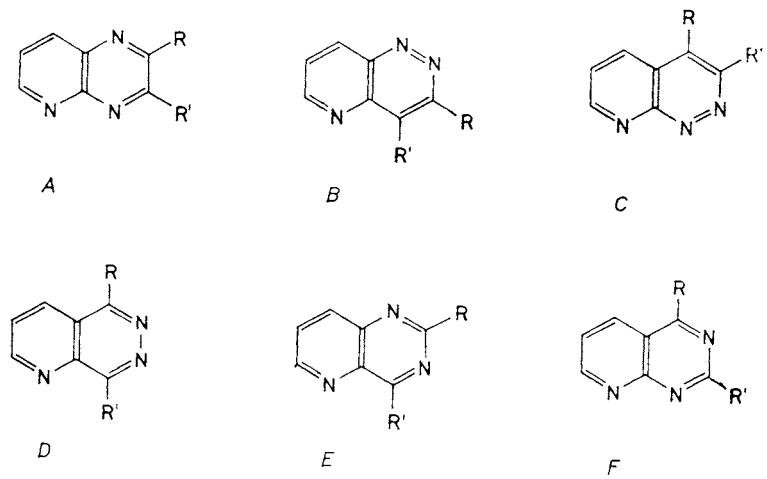
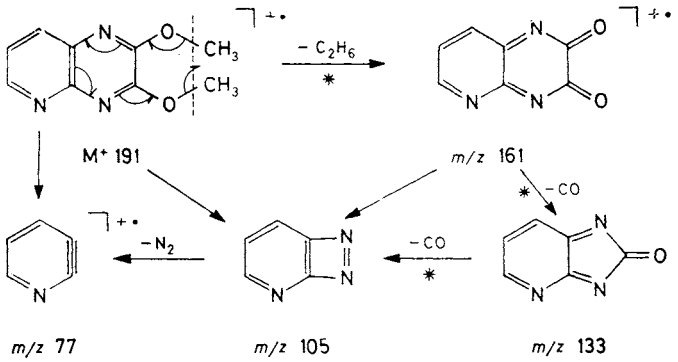


FIG. 1

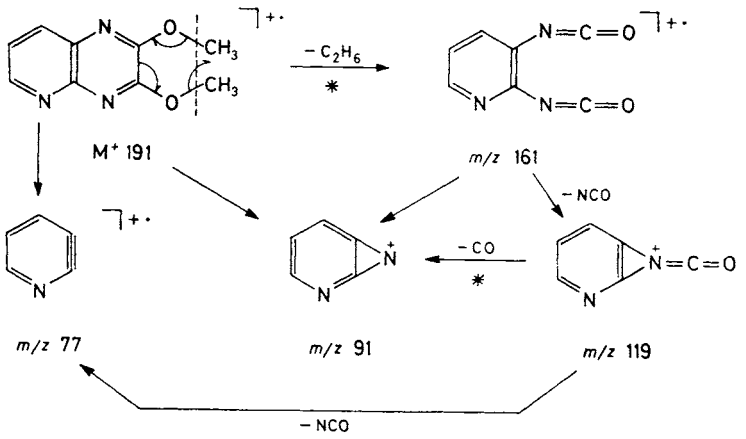
The structural types *A*–*F* considered for *I* and *II*

and an upfield shift of all remaining atoms $C_{(6)}$ to $C_{(10)}$ by −2·8 to −6·7 ppm (see Table II).

In the case of compound *II* it was evident from the above discussed arguments that it belongs to the same structural type A, but for a complete elucidation of the structure it was necessary to localize various substituents – methoxyl and isopropyl – on the pyrazine ring. Neither from the mass nor the NMR spectra of compound *II* unambiguous structural proof could be obtained. Therefore we tried to reduce the pyrazine ring of compound *II*. Hydrogenation on palladium catalyst and reduction with lithium aluminium hydride gave the same sole product – dihydro derivative – to which we assigned the structure *III* on the basis of NMR analysis. Hydrogenation of the double bond carrying the isopropyl group was confirmed by



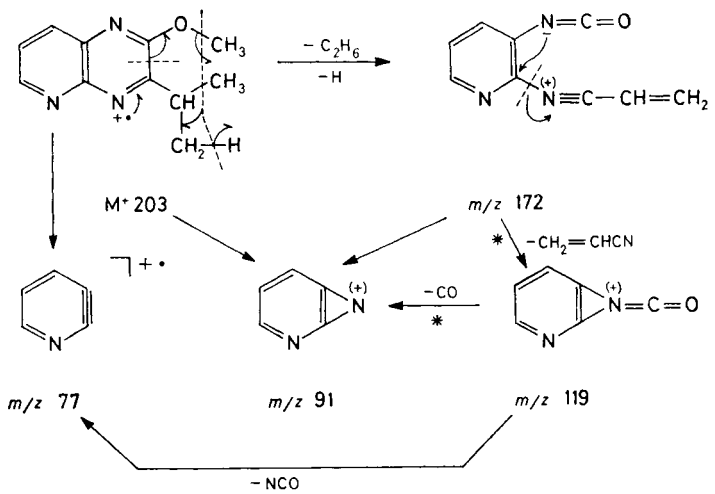
SCHEME 1



SCHEME 2

the coupling constant $J_{H,H}$ = 3·8 Hz of the isopropyl CH— proton with the newly introduced CH— atom of hydrogen of the pyrazine ring at δ 4·19. ^{1}H NMR spectrum in hexadeuteriodimethyl sulfoxide also permitted the demonstration of the interaction of this pyrazine hydrogen atom with the neighbouring NH proton (J = 2·4 Hz). However, for the localization of substituents the observation of the long-range coupling (J = 0·8 Hz) of the NH hydrogen atom (δ 6·95) with H-8 (δ 7·09) of the pyridine ring is of especial importance. This type of long-range couplings was observed, for example between the aromatic hydrogens H-4 and H-8 in pyrido[2,3-*c*]-pyridazine ($J_{4,8}$ = 1·0)[13], pyrido[3,2-*c*]pyridazine ($J_{4,8}$ = 0·75)[14] and between NH and H-4 hydrogens in indole ($J_{NH,4}$ = 0·8 Hz)[15,16]. In all the cases mentioned it is a planar W-type coupling pathway over five bonds. In our case this type of interaction is possible only between the $N_{(4)}$—H and H-8 atoms and it leads to the localization of the isopropyl into position 3. Hence, the product of reduction has the structure *III* and the native starting compound may be assigned analogously the structure of 2-methoxy-3-isopropylpyrido[2,3-*b*]pyrazine (*II*).

We also investigated the course of the reduction reactions in compound *I*. Although we used the methods described in literature for specific reductions of the pyrazine ring[2], applied successfully in other 2,3-disubstituted pyrido[2,3-*b*]pyrazines (for example dimethyl, diphenyl derivatives), we were unable to obtain the expected product from compound *I*. During catalytic hydrogenation in acetic acid only the pyridine ring was reduced under formation of 5,6,7,8-tetrahydro derivative *IV* (its structure was demonstrated by mass and NMR spectra, Table I and II). Hydrogenation in ethanol afforded the same product *IV*, but also derivative *V* in lesser amount,



SCHEME 3

in which the $N_{(5)}$-H atom was substituted by an ethyl group. Finally, the reduction of compound *I* with lithium aluminium hydride[5] took place under eliminatin of one molecule of methanol and the formation of monomethoxydihydro derivative *VI*. This is easily oxidized with air oxygen to methoxy derivative *VII* with the original heteroaromatic system. This was demonstrated by ^{1}H NMR spectra, when sample measured several days after the isolation of pure derivative *VI* already contained 13% of an oxidation product (*VII*) and the measurement repeated after a longer standing showed an increased content of compound *VII* (^{1}H NMR data for *VI* and *VII* see Table II). Summarily it may be stated that the reduction of pyrido[2,3-*b*]pyrazines substituted in position *2* or *3* with an alkoxy group proceeds in a way different from that in the case of an analogous substitution with an alkyl or aryl group.

It should be stressed that compounds *I* and *II* have not been isolated so far from any natural material, or prepared synthetically.

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