The Structures of Two New Glycine Conjugated Compounds from Cattle Urine

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Received December 19, 1973

Recently,^{1,2)} we have examined the acidic components of cattle urine by GLC and GC-MS; a new glycine conjugated compound, hexahydrohippuric acid, has been reported, together with hippuric and phenaceturic acids. In this communication, we would like to report the isolation and structural determination of two new glycine conjugated compounds, N-*n*-valerylglycine (I), $\text{CH}_3(\text{CH}_2)_5\text{CONHCH}_2\text{COOH}$, and N-*sec*-valerylglycine (II), $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)\text{CONHCH}_2\text{COOH}$, from the ether-soluble acidic fraction. These compounds were found in the urine of female Holstein Friesian cattle which received straw, soybean-curd refuse, ricebran, or even fresh green grass. Urine samples obtained from the cattle were used just after excretion, to avoid the possibility of degradation. Urine from pregnant cattle was not examined. Details of the samples are shown in Table I.

The ether-soluble acidic fraction was separated by the method previously reported.³⁾ A gas chromatogram of the methyl esters of the acidic compounds, using an SE-30 (25%)

FIG. 1. Gas Chromatogram of the Methyl Ester of the Ethyl Ether-soluble Acidic Fraction.

SE-30 (25%)/varaport 30 3 mm ϕ $\times$ 2.5 m, 80~250°C, 4°C/min, 0.8 kg/cm² H₂.

column, is shown in Fig. 1. The yields of the acidic fractions, both soluble in ether and insoluble in ether, and the percentage of peak 23 component are shown in Table II. The peak 23 product was isolated by preparative GLC. On hydrolysis with 10% aqueous sodium hydroxide solution, it gave an aqueous fraction, which was subjected to paper chromatography. A spot of glycine was detected by color reaction with ninhydrin. The peak 23 fraction was again subjected to GLC, with another column, PEG-20M (3%). Several peaks were detected in the gas-chromatogram; the two main peaks, peak 1 and 2, were isolated as colorless imbricate by preparative GLC. Yields: about 8 mg (peak I), 4 mg (peak 2)/liter of urine when extracted with ethyl acetate.

The methyl ester of the peak I compound, (II), gives MW 173 in the MS spectrum. $\text{C}_8\text{H}_{15}\text{O}_3\text{N}$ (173.21); IR $\nu_{\text{max}}^{\text{KBr}}$ 1250, 1755 (COOCH_3), 1650 cm^{-1} (CONH), 1170 and 1360 cm^{-1} ($\text{CH}_3\text{CH}_2(\text{CH}_3)\text{-C}$); NMR $\delta_{\text{Me}_4\text{Si}}^{\text{CDCl}_3}$ 0.98 (3H, d $J=7.2$ Hz, $\text{C-CH}(\text{CH}_3)\text{-C}$, 1.15

TABLE I. SAMPLES OF CATTLE URINE

Sample No. (Holstein Friesian)	1	2	3	4	5
Date of sampling	71.9.27	71.12.14	72.9.18	73.8.10	73.8.12
Age (sex)	3 (♀)	3 (♀)	5 (♀)	4 (♀)	4 (♀)
pH of urine	8.2	8.4	8.0	8.4	8.4
Specific gravity of urine	1.012	1.018	1.010	1.025	1.031
Cattle pasture and feed	a	a	a	b	b

a) Nishigamo, Kyoto (Ōmiya pasture) Feed; Rice straw, soybean-curd refuse, rice bran.

b) Ebetsu, Hokkaido (Rakuno College) Feed; Grass, assorted feed.

TABLE II. YIELDS OF ACIDIC FRACTION OF CATTLE URINE (g/liter URINE)
AND THE PERCENTAGE OF PEAK 23 COMPONENTS OF THE ETHYL ETHER-SOLUBLE
ACIDIC FRACTION FROM CATTLE URINE

Sample No.	1	2	3	4	5
Acidic part	2.57	2.14	2.49	4.12	4.01
soluble in ether	0.75	0.64	0.70	1.32	1.26
insoluble in ether	1.82	1.50	1.79	2.80	2.75
Components in peak 23 ^{a)}	7.29	12.85	11.57	6.91	7.50

^{a)} Calculated from gas-chromatogram areas, using an SE-30 (25%) column.

(3H, t $J=6.0$ Hz, CH_3CH_2), 1.68 (2H, m, $\text{CH}_3\text{CH}_2\text{CH}$), 3.68 (1H, m, $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)$), 3.77 (3H, s, COOCH_3), 4.07 (2H, d $J=4.4$ Hz, NHCH_2), 6.24 (1H, broad s, CONH). When D_2O was used for deuterium labeling, the signal at 6.24 ppm disappeared. In the MS spectrum of compound (II), peaks at 173, 145, 142, 114, 88, 85, 57, and 30 m/e are probably due to M, $\text{M}-(\text{CH}_2\text{CH}_2)$, $\text{M}-\text{OCH}_3$, $\text{M}-\text{COOCH}_3$, $\text{NHCH}_2\text{COOCH}_3$, $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)\text{CO}$, $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)$, and CH_3NH_2 ions, respectively.

The methyl ester of the peak 2 compound, (I), gives MW 173 in the MS spectrum. $\text{C}_8\text{H}_{15}\text{O}_3\text{N}$ (173.21); IR $\nu_{\text{max}}^{\text{KBr}}$ 1250, 1700 cm^{-1} (COOCH_3), 1600 cm^{-1} (CONH). In the MS spectrum of the compound (I), peaks at 173, 142, 131, 114, 88, 85, 57, and 30 m/e are probably due to M, $\text{M}-\text{OCH}_3$, $\text{M}-(\text{CH}_2\text{CH}:\text{CH}_2)$, $\text{M}-\text{COOCH}_3$, $\text{NHCH}_2\text{COOCH}_3$, $\text{CH}_3(\text{CH}_2)_3\text{CO}$, $\text{CH}_3(\text{CH}_2)_3$, and NH_2CH_2 , respectively.

The structures of (I) and (II) were confirmed by synthesis. Condensation of *n*-valeryl chloride with glycine methyl ester and

condensation of sec-valeryl chloride with glycine methyl ester in pyridine at room temperature gave the methylester of (I), colorless imbricate, mp 112~113°C, and the methylester of (II), colorless imbricate, mp 106~107°C, in yields of 54% and 49%, respectively. In both cases, the IR spectra and GLC retention times of the isolated methyl esters coincided with those of the synthetic compounds.

N-n-Valerylglycine and *N*-sec-valerylglycine are new glycine conjugated compounds found in animal urine, and are interesting for the light they throw upon metabolic pathways.

The authors wish to express their thanks to Mr. K. Sakata, Rakuno College, for his help in gathering the material.

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