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DEGRADATION OF CLAVULANIC ACID IN AQUEOUS ALKALINE SOLUTION

Jun Haginaka,*,[a] Hiroyuki Yasuda,[a] Toyozo Uno,[a] and Terumichi Nakagawa[b]
Faculty of Pharmaceutical Sciences, Mukogawa Women's University,[a] 4-16 Edagawa-cho, Nishinomiya 663, Japan and Faculty of Pharmaceutical Sciences, Kyoto University,[b] Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto 606, Japan

Potassium clavulanate was degraded in Na_2HPO_4 solution to yield 3-ethyl-2,5-di-(2-hydroxyethyl)-pyrazine, whose structure was elucidated by mass and NMR spectra.

KEYWORDS——clavulanic acid; alkaline degradation product; mass spectra; NMR spectra

In the previous paper,[1)] we described the degradation of clavulanic acid, z-(2R,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo-[3.2.0]-heptane-2-carboxylic acid, in aqueous solutions over a pH range of 3.15 to 10.10 at 35°C with ionic strength of 0.5. This communication reports the structure of the degradation product of potassium clavulante in weakly alkaline solution(pH 9.21).

potassium clavulanate was degraded in 0.1 M-Na_2HPO_4 solution at 100°C for 1 h to yield product(s) having UV-absorption maximum around 280 nm. The HPLC analysis of the solution shows one major and four minor peaks detected at 280 nm on a chromatogram(Fig. 1). We isolated the major peak substance by HPLC, and submitted it to mass and NMR spectral measurements.

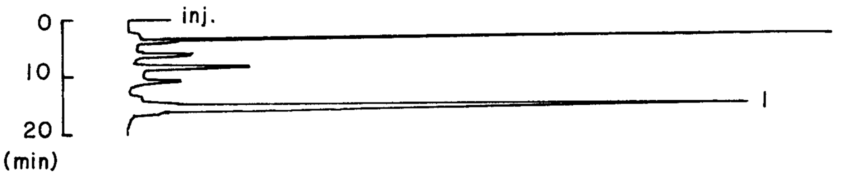
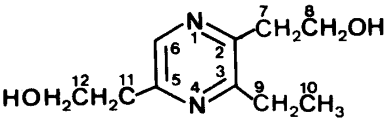


Fig. 1. Chromatogram of Degradation Product(s) of Clavulanic Acid

Potassium clavulanate(125 mg) was degraded in 0.1 M-Na_2HPO_4 solution(50 ml) at 100°C for 1 h. A 5 µl portion was introduced to HPLC under the following conditions: stationary phase; Develosil ODS-10(25 cm x 4.6 mm i.d.), mobile phase; H_2O/MeOH=3/1(v/v), sensitivity; 0.16 aufs, detection; UV-280 nm. 1; 3-ethyl-2,5-di-(2-hydroxyethyl)-pyrazine.



3-ethyl-2,5-di-(2-hydroxyethyl)-pyrazine(I)

Fig. 2. The Structure and Numbering of the Atom of the Isolated Product

The spectral data were reasonably elucidated as follows in accordance with the molecular structure of I(Fig. 2). Mass spectra[2]: m/z; 196(M^+, base peak)(obs. 196.1219, calc. 196.1212), 179(M^+ - OH), 178(M^+ - H_2O), 167(M^+ - CH_2CH_3), 166(M^+ - CHOH), 165(M^+ - CH_2OH), 135(M^+ - CHOH - CH_2OH). ^{1}H-NMR(in CD_3Cl)[3] δ: 1.30(3H, t, J = 7.1Hz, 10C-H), 2.86(2H, q, J = 7.1Hz, 9C-H), 3.04(4H, m, 7C-H and 11C-H), 3.8 - 4.1(2H, broad, OH), 4.11(4H, m, 8C-H and 12C-H), 8.21(1H, s, 6C-H). ^{13}C-NMR(in CD_3OD)[4] δ: 13.3(q, 10C), 28.4(t, 9C), 37.5(t, $11C^*$), 38.8(t, $7C^*$), 62.0(t, $12C^{**}$), 62.2(t, $8C^{**}$), 142.3(d, 6C), 151.4(s, $5C^{***}$), 153.2(s, $2C^{***}$), 157.9(s, $3C^{***}$), where (*), (**), and (***) indicate that the assignments are interchangeable. λ_{max}: 279 nm(in MeOH). Thus, the structure of the isolated product is determined as 3-ethyl-2,5-di-(2-hydroxyethyl)-pyrazine.

The degradation mechanism and the formation of other pyrazine derivatives will be discussed elsewhere.

REFERENCES AND NOTES

1) J. Haginaka, T. Nakagawa, and T. Uno, Chem. Pharm. Bull., 29, 3334 (1981).
2) Measured on JEOL mass spectrometer(JEOL Ltd., Tokyo, Japan) under the following conditions: ionizing energy 75 eV, acceleration voltage 8 kV, ionizing current 200 μA.
3) Measured on a JEOL FX-200 NMR spectrometer(JEOL). Tetramethylsilane was used as an internal reference.
4) Measured on a JEOL FX-200 NMR spectrometer(JEOL) employing the deutrium/frequency lock system. Tetramethylsilane was used as an internal reference.

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