100° for 4 hr. gave diacetylserratine (V), m.p. 212~214°, $C_{20}H_{29}O_5N$, IR, ν_{max} cm^{-1} : 1740 (C=O), no OH band, NMR : 8.51 (3H, s., $\geqslant C\text{-}CH_3$), 8.10 (3H, s., $-CO\text{-}CH_3$), 8.04 (3H, s., $-CO\text{-}CH_3$), 5.23 (1H, t., J=2.5 c.p.s., $>CH\text{-}OAc$). Hydrolysis of both (IV) and (V) regenerated serratine. In the NMR spectra below 6.5 τ, these two compounds revealed only one signal corresponding to one proton. This observation together with the τ values of the tertiary methyl group suggested the presence of $\begin{matrix}(C)\\(C)\end{matrix}\!\!>\!C\!<\!\!\begin{matrix}OH\\CH_3\end{matrix}$ system in the serratine molecule.

Dehydration of (IV) with $POCl_3$-pyridine at room temperature provided anhydromonoacetylserratine (VI), which was identified with an authentic sample of anhydromonoacetylserratinine II[2] derived from serratinine (I), by comparison of IR spectra, specific rotations and mixed melting point determination. From this result it is certain that serratine should be represented by the formula (III) with only the configuration at C-15 to be settled, since the structure and the full absolute stereochemistry of serratinine (I)[2] have been established.

Finally, the *cis* relationship between the C-13 and C-15 hydroxyl groups was shown by formation of serratine carbonate (VII), m.p. 295~297°, $C_{17}H_{23}O_4N$, IR, ν_{max} cm^{-1} : 1738 (C=O), no OH band, which was obtained by treatment of serratine with phosgene-pyridine at room temperature for one day.

From this steric relationship, the facile acetylation and hydrolysis of the tertiary hydroxyl group of serratine can be well explained by intramolecular transesterification through the cyclic intermediate as shown in the chart.

Consequently, serratine is represented by the absolute stereostructure of III.

Faculty of Pharmaceutical Sciences,
Osaka University,
Toyonaka, Osaka

Yasuo Inubushi (犬伏康夫)
Hisashi Ishii (石井 永)
Takashi Harayama (原山 尚)

Received October 21, 1966

[Chem. Pharm. Bull. 15(2) 252~253 (1967)]

UDC 547.964.4.08

Recovery of Biologically Active Peptides from Their 1-Dimethylaminonaphthalene-5-sulfonyl Derivatives

1-Dimethylaminonaphthalene-5-sulfonyl chloride (DNS-Cl), a reagent of fluorometry for amino compounds,[1] has been utilized for identification of minute quantities of amines and amino acids.[2] Recently, one of the authors applied this reagent to microanalysis of peptides whose identification was successfully achieved on thin-layer chromatogram.[3] We will report a new method to recover biologically active peptides from purified DNS-peptides by reductive elimination of DNS groups with metal sodium in anhydrous liquid ammonia at −70°.

Commercial colistin of 70% purity was treated with 50 folds of DNS-Cl in 50% acetone at pH 8.2. After standing for several hours at room temperature, the reaction

1) G. Weber : Biochem. J., **51**, 155 (1952).
2) W. R. Cray, B. S. Hartley : *Ibid.*, **89**, 59 (1963); Biochim. et Biophys. Acta, **21**, 58 (1956); N. Seiler, J. Wiechmann : Experientia, **20**, 559 (1964); *Ibid.*, **21**, 203 (1965).
3) Z. Tamura, J. J. Pisano, S. Udenfriend : Unpublished work.

mixture was extracted with chloroform, and the extract was chromatographed on a thin-layer of Silica gel H (developing solvent; Toluene-EtOH (3:1)). The main fluorescent band was eluted from the Silica gel with a mixture of MeOH, 28% NH_3 and pyridine (200:1:1) and dried *in vacuo*. The pure DNS-colistin thus obtained contained five DNS-groups in the molecule and had no antibacterial activity.

Two milligram of the DNS-colistin was dissolved in 2 ml. of tetrahydrofuran, added with 6 ml. of anhydrous liquid ammonia and 20 mg. of metal sodium at −70°, and stood for 10 minutes. The reaction was stopped by an addition of 200 mg. of ammonium acetate and the solution was evaporated to dryness. The residue was dissolved in 1.5 ml. of H_2O and pH was adjusted to 2~3 with *N*-AcOH and desalted through a Sephadex G-10 column.

The recovered colistin was identified by paper chromatography (Toyo-roshi No. 51A, developing solvent; *n*-BuOH-AcOH-H_2O (3:1:1) and *n*-BuOH-pyridine-AcOH-1% NaCl (30:20:6:24)) paper electrophoresis (Toyo-roshi No. 51A, HCOOH-AcOH-H_2O (28:20:52), 24 V./cm., 2 hours; 0.02*M* phosphate buffer, pH 7.2, 40 V./cm., 3 hours) and again by thin-layer chromatography of its DNS-derivative.

The purity of recovered colistin was determined as 98% or above from its specific activity against *E. Coli* (activity corresponding to 1 mg. of nitrogen; calcd. 15.6×10^4 unit, found 15.3×10^4 unit) and from ultraviolet absorption of its DNS-derivative (absorbance at 335 mμ corresponding 1 mole of nitrogen; Calcd. 255.2, Found 250.2). The overall recovery was 80~90%.

It has been already reported that tertiary peptides bonds are selectively cleaved by Birch-reduction,[4] and it might be expected that reductive elimination of DNS-groups from DNS-peptides with anhydrous liquid ammonia and metal sodium resulted in the cleavage of prolyl bonds in the peptides. Consequently the method was examined with commercial bradykinin.

DNS-bradykinin was similarly prepared and separated by thin-layer chromatography (developing solvent; *n*-BuOH-AcOH-H_2O (4:1:5), upper phase, added with 1% of mercaptoethanol). The purified DNS-bradykinin was eluted with a mixture of acetone, H_2O and pyridine (50:50:3) and dried *in vacuo*, dissolved in anhydrous liquid ammonia, then treated with metal sodium at −70°.

The recovered bradykinin was identified by thin-layer chromatography as its DNS-derivative (developing solvent; the same solvent as above and AcOMe-2-PrOH-28% NH_3 (9:7:4)). When 10~50 μg. of bradykinin was subjected to this method, the recovery was 63.6% by fluorometry of its DNS-derivative and about 60% by bioassay with Guinea-pig ileum. These coincidental values implied no racemization occurred during the procedure, although there would be some cleavage of the prolyl bond. Actually, when DNS-bradykinin was treated under the condition of Birch-reduction, the recovery of bradykinin was much reduced, while with penta DNS-colistin, any difference in recovery was observed.

The method presented will be useful for purification and analysis of minute quantities of peptides in biological materials.

Faculty of Pharmaceutical Sciences,
University of Tokyo, Bunkyo-ku,
Tokyo, Japan

Zenzo Tamura (田村善蔵)
Takenori Tanimura (谷村恿徳)
Hisanobu Yoshida (吉田久信)

Received November 8, 1966

4) M. Wilchek, S. Sarid, A. Pathornik : Biochim. Biophys. Acta, **104**, 616 (1965).