In the reaction with compound **1**, however, only 1.1 mol equivalent of TTN was enough for the completion. The mechanism for this case is, therefore, suggested as shown in Chart 3. In the use of 2.2 mol equivalents of TTN, the reaction seems to proceed under competition of both mechanisms shown in Chart 2 and 3, because of about half yield of diphenyl disulfide (see footnotes of Table I).

Previously, Ho and Wong[8] published dethioacetalization of 1,3-dithiacyclopentanes and 1,3-dithiacyclohexanes derived from several aldehydes or ketones with thallium (III) trifluoroacetate. The reaction proceeded in mild conditions and gave good yields of the parent carbonyl compounds. Our method, however, has the following advantages: (i) Thallium (III) trinitrate is much cheaper and more easily available than thallium (III) trifluoroacetate. (ii) The reaction mixture is generally colorless and transparent in our case, while it is brownish in their case. Hence, the end point of the reaction is more easily and clearly judged by the white precipitation of $TlONO_2$ in our method, compared with their observation of the end point to be milky white. (iii) Our method can be used for the compounds having functional groups (especially an aliphatic double bond) by the use of the suitable mol equivalent of the reagent. In their case, only very simple ketones and aldehydes were tested. (iv) The water of crystallization of TTN is available for the hydrolysis of the methyl acetals in the final step of the reaction.

Thus, this novel method will promise the increasing value of the ethylenedithioacetal function for the protection of the carbonyl group especially in the field of the syntheses of the complex natural products.

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Occurrence of Bufalitoxin, Cinobufotoxin and Their Homologs in Japanese Toad

The isolation and characterization of nine new bufotoxins from the skin of Japanese toad was described. They were efficiently separated and purified by high-speed liquid chromatography. The structures were elucidated to be bufalitoxin homologs (Ia—c), cinobufotoxin homologs (IIa—d), arenobufotoxin (III), and cinobufotalitoxin (IV) by degradative means.

In recent years three novel types of bufotoxins in which the succinoyl, adipoyl, and pimeloyl groups are displaced for the suberoyl residue of the so-called "bufotoxin,"[1-3] have been

1) H. Wieland and R. Alles, *Ber.*, **55**, 1789 (1922); H. Wieland, G. Hesse, and R. Hüttel, *Ann.*, **524**, 203 (1936); H. Wieland and H. Behringer, *ibid.*, **549**, 209 (1941).
2) H.O. Linde-Tempel, *Helv. Chim. Acta*, **53**, 2188 (1970).
3) K. Shimada, Y. Fujii, E. Mitsuishi, and T. Nambara, *Chem. Ind.* (London), **1974**, 342.

isolated from the skin of *Bufo vulgaris formosus* Boulenger.[4-6] In addition the existence of bufalin 3-sulfate,[7] cardenobufotoxin,[8] and sarmentogenin 3-sulfate[9] in the Japanese toad has also been demonstrated. Now the isolation and characterization is reported of nine new bufotoxins including bufalitoxin, cinobufotoxin, and their homologs, from the skin of Japanese toad.

The ethanolic extract of the skin obtained from 1800 toads was chromatographed repeatedly in the manner as previously reported[3,4] to give a bufotoxin fraction which exhibited a single spot on the thin-layer chromatogram. This fraction, however, was separated into five peaks by high-speed liquid chromatography on a μ-Bondapak C_{18} column (Waters Associates Inc., Milford) using MeOH–H_2O (2: 1) as a solvent. Each fraction was further purified by high-speed liquid chromatography and gel filtration on Sephadex LH-20 to provide four new bufotoxins as colorless amorphous substances. All these compounds showed a positive result with Sakaguchi's reagent and a negative test with ninhydrin. Hydrolytic cleavage with 6N hydrochloric acid yielded arginine which was identified by thin-layer chromatography. These results indicated the presence of a peptide bond involving the α-amino group of arginine. Upon enzymatic hydrolysis with a hog pancreas lipase preparation (Sigma Chemical Co., St. Louis), followed by methylation with diazomethane bufotoxins yielded bufogenin 3-hemidicarboxylate methyl esters which were unequivocally identified by direct comparison with the authentic samples, respectively. The usual criteria, *i.e.* elemental analyses and nuclear magnetic resonance spectral data justified to assign the structures bufalitoxin (Ia),[10] mp 200—205° (decomp.), $[\alpha]_D^{21}$ −5.5° (c=0.09 in MeOH), bufalin 3-pimeloylarginine ester (Ib), mp 205—206.5° (decomp.), $[\alpha]_D^{21}$ −5.5° (c=0.18 in MeOH), bufalin 3-adipoylarginine ester (Ic), mp 210—213° (decomp.), $[\alpha]_D^{22}$ +5.5° (c=0.09 in MeOH), and arenobufotoxin (III),[12] mp 182—184° (decomp.), $[\alpha]_D^{23}$ −33.3° (c=0.09 in MeOH), to new bufotoxins. In a previous paper we reported the isolation of bufalin 3-succinoylarginine ester (Id),[6] and therefore the presence of a series of bufalitoxin homologs (Ia—d) in the Japanese toad has been disclosed.

Another bufotoxin fraction obtained by partition chromatography[3,4] was divided into five peaks by high-speed liquid chromatography under the same conditions as described above. Further purification of each fraction gave five new bufotoxins. Among these cinobufagin 3-succinoylarginine ester (IId), mp 200—202.5°, $[\alpha]_D^{15}$ +27.8° (c=0.11 in $CHCl_3$), was afforded as colorless prisms (from MeOH). The remaining new bufotoxins, cinobufotoxin (IIa),[14] mp 170—175° (decomp.), $[\alpha]_D^{16}$ +5.6° (c=0.09 in MeOH), cinobufagin 3-pimeloylarginine ester (IIb), mp 175—178° (decomp.), $[\alpha]_D^{16}$ +6.3° (c=0.08 in MeOH), cinobufagin 3-adipoylarginine ester (IIc), mp 183—186° (decomp.), $[\alpha]_D^{17}$ +4.7° (c=0.11 in MeOH), and cinobufotalitoxin (IV), mp 176—178° (decomp.), $[\alpha]_D^{16}$ −5.4° (c=0.09 in MeOH), were obtained as colorless amorphous substances. The structures were similarly elucidated by degradative means.

The physiological activity of these new bufotoxins will be the subject of a future communication. Further studies on the isolation of the new bufotoxin from the Japanese toad

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7) K. Shimada, Y. Fujii, and T. Nambara, *Tetrahedron Letters*, **1974**, 2767.
8) Y. Fujii, K. Shimada, Y. Niizaki, and T. Nambara, *Tetrahedron Letters*, **1975**, 3017.
9) Y. Fujii, K. Shimada, and T. Nambara, *Chem. Ind.* (London), "in press".
10) Bufalitoxin was synthesized by Pettit, *et al.*[11]
11) G.R. Pettit and Y. Kamano, *Chem. Commun.*, **1972**, 45.
12) Arenobufotoxin was first isolated from the toad venom by Chen, *et al.*,[13] but its purity and structure still remained uncertain.
13) K.K. Chen, H. Jensen, and A.L. Chen, *J. Pharmacol. Exptl. Therap.*, **43**, 13 (1931); K.K. Chen and A.L. Chen, *ibid.*, **49**, 514, 529 (1933).
14) Cinobufotoxin was isolated from the Japanese toad by Ohno, *et al.*, but its complete structure has not yet been elucidated.[15]
15) S. Ohno and M. Komatsu, *Yakugaku Zasshi*, **73**, 651, 796 (1953).

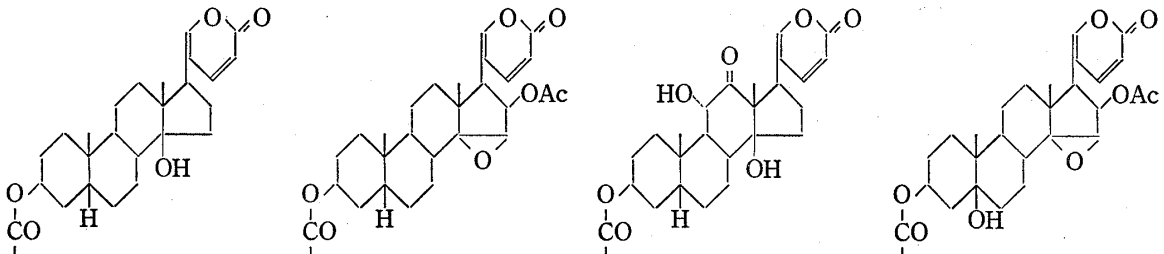

Chart 1

are being conducted in these laboratories and the details will be reported in the near future.

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Fukinotoxin, a New Pyrrolizidine Alkaloid from *Petasites japonicus*[1)]

Fukinotoxin (I), a novel pyrrolizidine alkaloid, was isolated from young scape of *Petasites japonicus* and its structure has been shown to be (12*R*,13*R*,15*R*,3'*R*)-12-hydroxy-4,12,13-trimethyl-4,8-secosenec-1-enine-15-spiro-2'-(3'-methyl)oxiran with the aid of chemical and X-ray crystallographic analyses.

In the course of the studies on carcinogenic activity of pyrrolizidine alkaloids in Compositae plants,[2)] a new alkaloid, named fukinotoxin (I) which has highly cytotoxic activity,[3)] was isolated from young scape of *Petasites japonicus* MAXIM. (Japanese name: Fuki-no-toh). The present paper deals with the structural determination of (I) by discussing the result of chemical study and X-ray analysis.

(I) was isolated from the MeOH extract of the young scape using silica gel column chromatography and showed mp 129.0—131.0° (from acetone), $[\alpha]_D$ +63.8° ($CHCl_3$), $[\theta]^{21°}_{max}$ (methylcyclohexane) +12900(239 nm), +17500 (278 nm),[4)] OH(3300 cm^{-1}), and CO(1735 cm^{-1}, broad)

1) Part VI in the series "Studies on Constituents of Crude Drug". For Part V see 2).
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