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20,21-Epoxyresibufogenin, a Novel Bufogenin isolated from Bufonis Venenum

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From a crude drug, toad cake (Bufonis Venenum), a new bufogenin was isolated and its structure was elucidated as 20,21-epoxyresibufogenin by spectral analysis, mainly by ^{13}C-NMR spectroscopy.

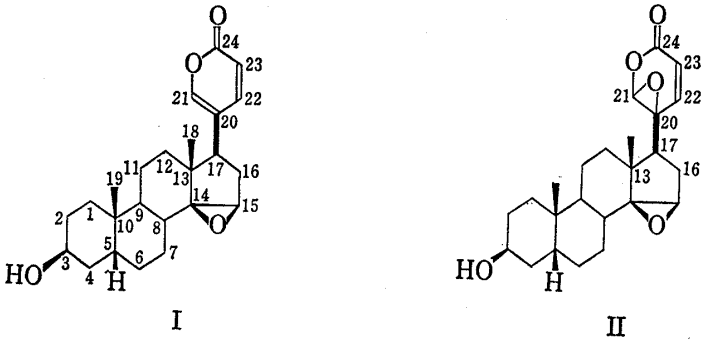
Keywords——Bufonis Venenum; toad cake; amphibian, steroid; bufogenin; resibufogenin; epoxide; structure; ^{13}C-NMR

To identify and quantify a crude drug, Bufonis Venenum (Ch'ang Su, toad cake), in mixtures of several crude drugs, *i.e.* Chinese prescriptions, characteristic compounds of toad cake were investigated.

Commercial toad cake was extracted with chloroform to give an extract, which was chromatographed on silica gel with a mixture of benzene–acetone (7: 1). Apart from resibufogenin (I), the main constituent, a new bufogenin (II), mp 197—202°, $[\alpha]_D^{22}$ +50° (EtOH) was separated as colorless needles. This compound has the composition $C_{24}H_{32}O_5$, MS *m/z*, 400 (M^+), 382 ($M^+ - H_2O$), 356 ($M^+ - CO_2$) and has UV max at 225 nm (inflection). The existence of II had been indicated by thin-layer chromatography (TLC), but it has not been isolated yet.[1] The coexistence of II with I and the elemental analysis data suggest that the two compounds are closely related. Thus, the spectra of II were compared with those of I.

In the ultraviolet (UV) spectrum of II, the strong absorption at 300 nm in I was no longer present, which implied change of the lactone-ring system of I. In the (IR) spectrum of II, the 1720 cm^{-1} band of I was shifted to 1740 cm^{-1}, which also implied some change near the carbonyl group.

The proton magnetic resonarce (PMR) spectrum of II was compared with that of I, which has already been assigned.[2] The PMR spectrum of II exhibited two angular methyl signals and one singlet proton (1H) signal at δ 3.52, which can be assigned to epoxide proton (15-H) by analogy with compound I. Compound II also showed a broad singlet signal (1H) at δ 4.09 attributable to 3-α-H. However, the olefinic proton region of II was quite different form that of I. Doublet signals centered at 7.34 in I (21-H) were completely absent from this



I II

Chart (Structure)

Table I. PMR Chemical Shifts of I and II (in $CDCl_3$)

	I	II
18-Me	0.79 (3H, s)	0.97 (3H, s)
19-Me	1.00 (3H, s)	1.01 (3H, s)
15-H	3.59 (1H, s)	3.52 (1H, s)
3-H	4.18 (1H, br. s)	4.09 (1H, br. s)
21-H	7.34 (1H, d, J 3 Hz)	5.26 (1H, s)
23-H	6.30 (1H, d, J 10 Hz)	5.94 (1H, d, J 10 Hz)
22-H	7.87 (1H, dd, J $^{10\ \mathrm{Hz}}_{3\ \mathrm{Hz}}$)	7.85 (1H, d, J 10 Hz)

region in II, and a new singlet appeared at δ 5.26 in the spectrum of II. A pair of double bond protons coupled to each other (J=10 Hz) (22, 23-H) were still observed in II. These results suggest that II has another epoxide ring at C-20/C-21 on the lactone ring of I (Table I).

To confirm this, ^{13}C-NMR spectra of both compounds were measured in $CDCl_3$. As the assignment of carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of I has not been reported, as far as we know, we began with the assignment of I.

The A to C ring skelton is common to cardenolide, the spectrum of which has already been reported.[3] Each carbon on these ring was readily assigned. Epoxide and neighboring carbons were characterized with the aid of literature data.[4] The lactone ring carbons, C-23 (δ 115.1), C-21 (δ 149.6) and C-22 (δ 147.1) were assigned by the proton selective decoupling of 23-H (δ 6.30), 21-H (δ 7.34) and 22-H (δ 7.87), respectively. The remaining two singlets were assigned readily to C-20 (δ 122.3) and C-24 (δ 162.0) on the basis of chemical shift considerations (Table II).

Table II. ^{13}C-NMR Chemical Shifts of I and II (in $CDCl_3$)

	I	II		I	II
1	29.5 (t)	29.5 (t)	13	45.2 (s)	44.1 (s)
2	27.9 (t)	27.9 (t)	14	74.7 (s)	75.2 (s)
3	66.7 (d)	66.6 (d)	15	59.8 (d)	59.8 (d)
4	33.3 (t)	33.2 (t)	16	32.4 (t)	28.6 (t)
5	35.9 (d)	35.9 (d)	17	47.8 (d)	51.7 (d)
6	25.8 (t)	25.8 (t)	18	16.8 (q)	16.2 (q)
7	20.8*(t)	20.7 (t)	19	23.7 (q)	23.7 (q)
8	39.3 (d)	39.2 (d)	20	122.3 (s)	56.5 (s)
9	33.6 (d)	33.2 (d)	21	149.6 (d)	84.6 (d)
10	35.5 (s)	35.5 (s)	22	147.1 (d)	147.9 (d)
11	21.1*(t)	20.7 (t)	23	115.1 (d)	121.3 (d)
12	39.3 (t)	39.7 (t)	24	162.0 (s)	159.8 (s)

Letters in parentheses indicate the multiplicity of the peaks (off-resonance decoupled spectrum); s: singlet, d: doublet, t: triplet, q: quartet
*) These assignments may be reversed.

The spectrum of II was then compared with that of I. From C-1 to 19, each carbon signal of II corresponded to that of I. With the exceptions of C-13 ,16 and 17, chemical shift differences of corresponding carbons are less than 0.6 ppm. This supports the view that II has the same carbon skelton (at least in the A, B and C rings) as I. A downfield shift (3.9 ppm) of C-17 and upfield shifts of C-13 and C-16 (1.1 and 3.8 ppm, respectively) were observed, which suggested the introduction of a functional group at the β-position relative to C-17, and at the γ-position relative to C-13 and C-16. The only suitable position of substitution was C-20. Elemental analysis and MS allowed one more oxygen in II, so the possible structure for II was restricted to 20,21-epoxyresibufogenin. The ^{13}C-NMR signals of C-20—24 are consistent with this structure. As in the PMR spectrum, only one pair of olefinic carbon signals

(δ 147.9 and 121.3) was observed, and these signals were easily assigned to C-22 and C-23, respectively. The lactone carbonyl signal (δ 159.8) was not very different from that of I. There was a marked change compared with I, which gave another pair of double bond carbon signals. In the spectrum of II, no other olefinic carbon signals were seen, but new signals appeared at δ 56.5 (s) and 84.6 (d). The former was a typical epoxide carbon as judged by its chemical shift,[4] and the latter signal was consistent with C-21, a two-oxygen-substituted carbon, in view of its chemical shift and multiplicity. The stereochemistry at C-20 is under investigation.

As far as we know, this compound is the first example of a bufogenin which has two epoxide rings in the molecule at unusual positions. Testing of II for biological activity is in progress.

Experimental

Isolation of II——Commercial toad cake (Bufonis Venenum, J.P.IX) from Tokyo Market (100 g) was exhaustively extracted with $CHCl_3$ in a Soxhlet extractor to obtain an extract (*ca.* 25 g), which was chromatographed on Si gel with a mixture of C_6H_6–Me_2CO (7: 1). Just before the elution of resibufogenin (I), a new compound (II) (100 mg) was obtained as colorless needles, mp 197—202°. Upon TLC on a precoated Si gel plate (Merck 5554), developed with C_6H_6–Me_2CO (5: 2) followed by spraying H_2SO_4 and heating, II appeared as a reddish-brown spot at *Rf* 0.52,[1] while I was a dark-green spot at *Rf* 0.41. Compound II gave $[\alpha]_D^{22}$ +50° (EtOH, c=0.02), *Anal.* Calcd for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found: C, 71.82; H, 8.39. IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 1740, 1120, 1080. UV λ_{max}^{EtOH} cm^{-1} 225 nm (inflection). MS *m/z*: 400 (M^+), 382 (M^+-H_2O), 356 (M^+-CO_2). PMR and ^{13}C-NMR: See text.

^{13}C-NMR Measurement——The spectrum of each compound (50 mg) in $CDCl_3$ (1 ml) (10 mm tube) was recorded at 25° with TMS as an internal standard. The spectrometer (JEOL PFT-100) was operated at 25.15 MHz, with a 16 μsec (45°) pulse every 3.0 sec (1000 scans) and 8W of wideband r.f. for ^{1}H decoupling. It was connected to an EC-6 computer (8192 data points for 5 kHz). Where appropriate, assignments were confirmed by off-resonance decoupling, selective decoupling or a PRFT experiment.

References and Notes

1) M. Komatsu, Y. Kamano, and M. Suzuki, *Japan Analyst*, **14**, 1049 (1965).
2) Y. Kamano, H. Yamamoto, Y. Tanaka, and M. Komatsu, *Tetrahedron Lett.*, **1968**, 5673.
3) K. Tori, H. Ishii, Z.W. Wolkowski, C. Chachaty, M. Sangaré, F. Piriou, and G. Lukacs, *Tetrahedron Lett.*, **1973**, 1077.
4) D.R. Paulson, F.Y.N. Tang, G.F. Moran, A.S. Murray, B.P. Pelka, and E.M. Vesquez, *J. Org. Chem.* **40**, 184 (1975).

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New Methods and Reagents in Organic Synthesis. 9.[1] C-Acylation of Nitromethane with Aromatic Carboxylic Acids using Diethyl Phosphorocyanidate (DEPC)

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Aromatic α-nitroketones can be conveniently prepared from aromatic carboxylic acids and nitromethane by the action of diethyl phosphorocyanidate (DEPC) in the presence of triethylamine.