[Chem. Pharm. Bull. 24(8)1714—1717(1976)]

UDC 547.947.09 : 615.324.015.076.9

Studies on the Surface Anesthetic Activity of Bufadienolides Isolated from Ch' an Su

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(Received September 16, 1975)

The surface anesthetic activities of ten bufadienolides, *i.e.*, resibufogenin, bufalin, bufotalin, desacetyl-bufotalin, cinobufagin, desacetyl-cinobufagin, cinobufotalin, desacetyl-cinobufotalin, telocinobufagin and marinobufagin, were studied using the guinea pig cornea. With exceptions of resibufogenin and marinobufagin, all substances tested were more effective than cocaine. Their anesthetic activities were in the following order: bufalin>telocinobufagin>bufotalin>cinobufagin>cinobufotalin>desacetyl-bufotalin≧desacetyl-cinobufagin≧desacetyl-cinobufotalin. Resibufogenin and marinobufagin had no appreciable effect on the cornea. There is some correlation between the chemical structure and the surface anesthetic activity in the bufadienolides tested.

Recently, about thirty bufadienolides having cardiotonic activity were purely isolated from the Chinese drug Ch' an Su.[2–4] This drug is called "Senso" in Japanese and is prepared from the skin of the native toad such as *Bufo bufo gargarizans*, *Bufo bufo metanostrictus*, *etc.* These bufadienolides have been studied by many investigators from the viewpoints of pharmacology and chemistry. Chen[5] has reported a systematic study of the relationship between their chemical structures and cardiotonic activities.

With respect to pharmacological effects other than cardiotonic activity of bufadienolides, it is well known that the bufadienolides have the surface anesthetic activity. Okada and Ishihara[6] demonstrated that bufalin was the bufadienolide having the most powerful surface anesthetic activity among the active constituents of Ch' an Su. However, no systematic investigation of surface anesthetic activity of bufadienolides has been presented excepting a study on several bufadienolides.[7]

The present investigation was undertaken to elucidate a systematic relationship between the chemical structure and the surface anesthetic activity of bufadienolides.

Materials and Methods

Surface Anesthetic Activity——Method was similar to that described by Chance and Lobstein.[8] Male guinea pigs weighing approximately 450 g were previously tested the normal reflexes of the cornea to pin-pricks exerted by a mandrin of a 1/4 gauge needle. After the application of three drops of the test solution through a 1/4 gauge needle to the cornea, eyes were rested closed for one minute and then the remainder was wiped out with a piece of absorbent cotton. The test of six pricks was applied every 5 min for 30 min. The number of times the pricks fails to elicit a response during the 30-min period was added up and the sum, out of a possible 36, gave an indication of the degree of anesthesia.

Acute Toxicity——Male dd mice weighing 15—18 g were used. Test samples dissolved in 50% propylene glycol in distilled water were injected in the caudal vein. Appearance of toxicities of the samples was ob-

1) Location: *Yayoi-cho, Chiba, Japan*; a) *Temp, Arizona, U.S.A.*
2) M. Komatsu, Y. Kamano, and M. Suzuki, *Japan Analyst*, **14**, 1049 (1965).
3) M. Komatsu and T. Okano, *Japan Analyst*, **15**, 1115 (1966).
4) M. Komatsu and T. Okano, *Yakugaku Zasshi*, **87**, 712 (1967).
5) K.K. Chen, *J. Med. Pharm. Chem.*, **3**, 111 (1961).
6) M. Okada and T. Ishihara, *Itsuu Kenkyusho Nempo*, **8**, 66 (1957).
7) M. Okada, T. Suga, and S. Matsumoto, *Itsuu Kenkyusho Nempo*, **11**, 75 (1960).
8) M.R.A. Chance and H. Lobstein, *J. Pharmacol. Exptl. Therap.*, **82**, 203 (1944).

served for 48 hr and the LD_{50} values with 95% confidence limits were calculated by the method of Litchfield-Wilcoxon.[9]

Drugs——Bufadienolides tested here were bufalin, bufotalin, desacetyl-bufotalin, desacetyl-cinobufotalin, resibufogenin and marinobufagin. All compounds were dissolved in 25% propylene glycol. Cocaine hydrochloride (Sankyo) was used as a standard drug.

Results

Surface Anesthetic Activity

As is evident from Fig. 1, log.dose-response curves of bufadienolides except resibufogenin and marinobufagin were generally proportional to that of cocaine hydrochloride and these bufadienolides were more effective than cocaine. According to ED_{50} values (Table I) the surface anesthetic activity of bufadienolides was in the following order: bufalin>telocinobufagin>bufotalin>cinobufagin>cinobufotalin>desacetyl-bufotalin≧desacetyl-cinobufagin≧desacetyl-cinobufotalin. Resibufogenin and marinobufagin had no appreciable effect on the corneal reflex in a concentration of 0.05%, and the higher concentrations were not tested because of their insolubilities.

Propylene glycol used as a solvent in the present study had no appreciable effect on the corneal reflex.

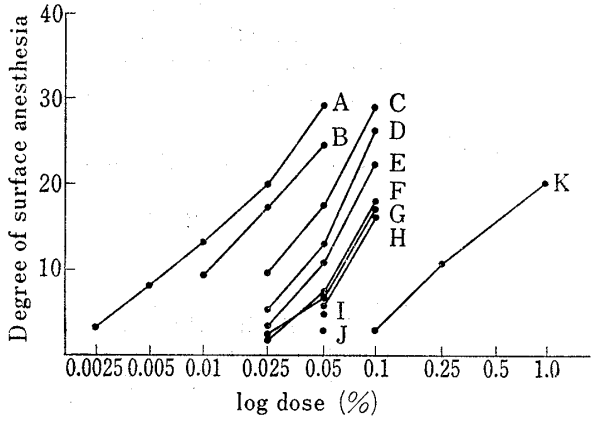


Fig. 1. log Dose–Response Curves of Bufadienolides

A, bufalin; B, telocinobufagin; C, bufotalin; D, cinobufagin; E, cinobufotalin; F, desacetyl-bufotalin; G, desacetyl-cinobufagin; H, desacetyl-cinobufotalin; I, marinobufagin; J, resibufogenin; K, cocaine hydrochloride

Acute Toxicity

As shown in Table I, the acute toxicity of bufadienolides in mice was in the following order: bufalin>cinobufagin>marinobufagin>bufotalin>telocinobufagin>cinobufotalin>resibufogenin>desacetyl-bufotalin>desacetyl-cinobufagin>desacetyl-cinobufotalin.

As toxic symptoms of these bufadienolides, mice showed sedative state followed by tremor, clonic and tonic convulsions and

Table I. Surface Anesthetic Activities of Bufadienolides Estimated by the Corneal Reflex of Guinea Pigs and Acute Toxicities in Mice

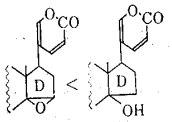
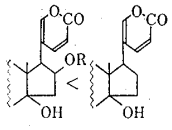
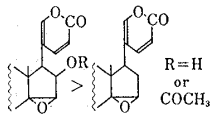
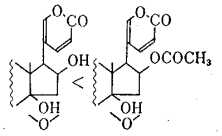
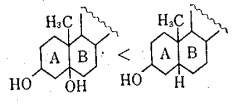
Substance	ED_{50} (%) (mM/liter)	Potency[a] ratio	LD_{50} (mg/kg, *i. v.*)
Bufalin	0.0166(0.429)	42.7	0.74(0.68— 0.82)
Telocinobufagin	0.0276(0.686)	25.6	4.34(3.95— 4.77)
Bufotalin	0.0513(1.186)	13.8	4.13(3.77— 4.52)
Cinobufagin	0.0645(1.498)	11.0	1.21(1.09— 1.33)
Cinobufotalin	0.0760(1.702)	9.3	5.15(4.77— 5.56)
Desacetyl-bufotalin	0.1000(2.484)	7.1	10.0 (9.1 —11.0)
Desacetyl-cinobufagin	0.1000(2.490)	7.1	15.0(13.4 —16.8)
Desacetyl-cinobufotalin	0.1270(3.041)	5.6	>20.0
Resibufogenin	—	—	5.68(5.44— 5.91)
Marinobufagin	—	—	2.73(2.31— 3.22)
Cocaine hydrochloride	0.7090	1.0	—

a) ED_{50}(%) of bufadienolides / ED_{50}(%) of cocaine-HCl

9) J.T. Litchfield Jr. and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).

finally, died after repeated spasms as reported by Okada, *et al.*[10] When these compounds were injected intravenously, the foregoing symptoms were very transient and died usually within one hour.

Table II. Relationships between Chemical Structure and Surface Anesthetic Activity of Bufadienolides

With regard to the ring D Resibufogenin<Bufalin Cinobufagin<Bufotalin Marinobufagin<Telocinobufagin Desacetyl-cinobufagin≦Desacetyl-bufotalin	
Desacetyl-bufotalin<Bufalin Bufotalin<Bufalin	
Resibufogenin<Desacetyl-cinobufagin Marinobufagin≦Desacetyl-cinobufotalin Resibufogenin<Cinobufagin Marinobufagin<Cinobufotalin	
Desacetyl-bufotalin<Bufotalin Desacetyl-cinobufotalin<Cinobufotalin Desacetyl-cinobufagin<Cinobufagin	
With regard to the rings A and B Telocinobufagin<Bufalin Cinobufotalin<Cinobufagin Desacetyl-cinobufotalin≦Desacetyl-cinobufagin (Marinobufagin≧Resibufogenin)	

Discussion

The surface anesthetic activities of the bufadienolides were studied and compared with that of cocaine hydrochloride. There is some correlation between the chemical structure and the surface anesthetic activity in the ten bufadienolides tested. Table II indicates the structure-activity relationships. Regarding the ring D on the steroid molecular structure, the following assumptions can be made: 1) Oxidation of the 14β-hydroxy group to a 14β,15β-epoxy group decreases the potency (4 instances with no exception); 2) Introduction of 16β-hydroxy or -acetoxy group decreases the potency of 14β-hydroxy derivatives (2 instances with no exception) whereas this modifications increase the potency of 14β,15β-epoxy derivatives (4 instances with no exception); 3) Acetylation of the 16-hydroxy group increases the potency (3 instances with no exception); 4) Regarding the ring A and B, introduction of hydroxy group in the position 5β decreases the potency (3 instances).

Surface anesthetic activity of these bufadienolides is considered to be not always proportional to the acute toxicity (Table I).

Tamm[11] has reported that introduction of an epoxy group in the position 14β and 15β greatly decreases the cardiotonic activity, but never completely loses, and that acetylation of the 16β-hydroxy group of the 14β,15β-epoxy derivatives increases the potency, and that

10) M. Okada, T. Suga, H. Takabori, T. Ishihara, and H. Ogura, *Asian Med. J.*, 3, 5 (1960).
11) Ch. Tamm, "Proceedings of the First International Pharmacological Meeting," Vol. 3, Pergamon Press, Inc., London, 1963, p. 11.

hydroxylation at C-16 decreases the potency. From the results of the present study, together with those reported by Tamm,[11] there is a definite correlation between the anesthetic activity and the cardiotonic activity. As mentioned by Chen,[5] deacetylation of the 16-acetoxy group results in a loss or reduction of cardiotonic activity, *i.e.*, desacetyl-derivatives of cinobufotalin and cinobufagin were inactive on the cat heart. In the present paper, the anesthetic activities of 16-desacetyl-derivatives of bufotalin, cinobufotalin and cinobufagin were smaller than those of the parent compounds.

On the other hand, Okada, *et al.*[7] did not support the parallelism between the surface anesthetic activity and the cardiotonic activity by reason that on the frog heart gamabufotalin was something more effective than bufalin, but on the rabbit cornea the former was less potent than the latter by a factor of about one twentieth.

Acknowledgement Gratitude is due to Prof. H. Kitagawa and Dr. T. Sato, Faculty of Pharmaceutical Sciences, University of Chiba, for helpful suggestions regarding the manuscript.