Tetrahedron Letters No.54, pp. 5673-5676, 1968. Pergamon Press. Printed in Great Britain.

THE ISOLATION AND STRUCTURE OF NEW BUFADIENOLIDES, 3-(HYDROGEN SUBERATES) OF RESIBUFOGENIN, CINOBUFAGIN AND BUFALIN THE STRUCTURE OF THE SO-CALLED "BUFOTOXINS" [1]

Yoshiaki Kamano, Hiroshi Yamamoto, Yoshihiro Tanaka and Manki Komatsu

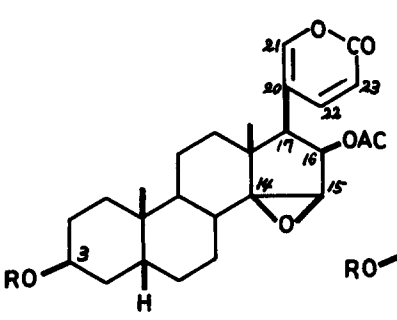
Research Laboratory, Taisho Pharmaceutical Co., Ltd., Tokyo, Japan

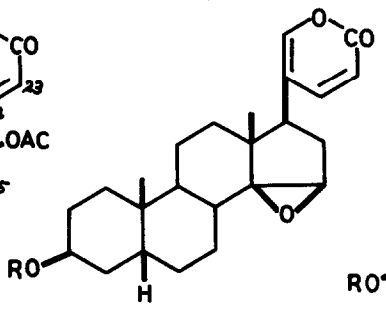
(Received in Japan 10 August 1968; received in UK for publication 30 September 1968)

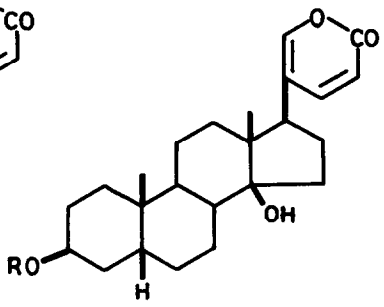
This paper describes on the isolation from Ch'an Su[2] of three new bufogenin esters, which have been elucidated to be cinobufagin 3-(hydrogen suberate) (II), resibufogenin 3-(hydrogen suberate) (V) and bufalin 3-(hydrogen suberate) (VIII). The location of suberoyl-arginine residue on the so-called "bufotoxins" will be also discussed.

The chloroform extract of Ch'an Su was subjected to chromatography on silica gel, which was eluted with an n-hexane-acetone mixture by adopting the dry method[3]. A mixture of the bufogenin 3-(hydrogen suberates) thus isolated was then separated into three fractions (II, V and VIII) by carrying out careful rechromatography as above.

Compound II, mp. 138-140°, was obtained as colorless needles from ethyl acetate and had the formula $C_{34}H_{46}O_9$. The compound had following spectral properties; λ_{max}^{EtOH} 296 mμ (log ε 3.51); ν_{max}^{KBr} 3500-3100 cm^{-1} (OH), 3040 cm^{-1} (C_{15}-H), 1745-1720 cm^{-1} (lactone and ester CO), 1690 cm^{-1} (carboxyl CO), 1635-1620, 1540 cm^{-1} (C=C of α-pyrone ring), 1240 cm^{-1} (C-O); τ (in $CDCl_3$) 1.99 (1H, dd, J = 10.0 and 2.5 cps, C_{22}-H), 2.75 (1H, d, J = 2.5 cps, C_{21}-H), 3.22 (1H, d, J = 10.0 cps, C_{23}-H), 4.47 (1H, d, J = 9 cps, C_{16}-H),[4] 4.84 (1H, broad peak, C_3-H), 6.30 (1H, s, C_{15}-H), 7.80 (1H, d, J = 9 cps, C_{17}-H),[4] 8.98 (3H, s, 19-CH_3), 9.17 (3H, s, 18-CH_3). These data support the presence of α-pyrone ring, C_{16}-acetoxyl and $C_{14,15}$ β-epoxy groupings, thus indicating that the structure of II is closely related with that of cinobufagin (I)[5]. While I had a C_3-proton signal at τ 5.87 and had an alcoholic hydroxyl







I. R = H cinobufagin

II. R = -CO-$(CH_2)_6$-COOH

III. R = -CO-$(CH_2)_6$-$COOCH_3$

IV. R = H resibufogenin

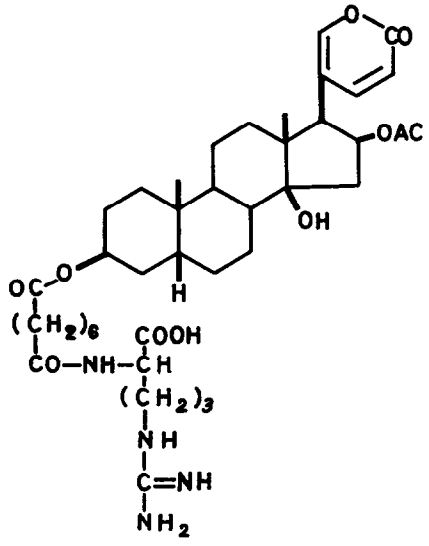
V. R = -CO-$(CH_2)_6$-COOH

VI. R = -CO-$(CH_2)_6$-$COOCH_3$

VII. R = H bufalin

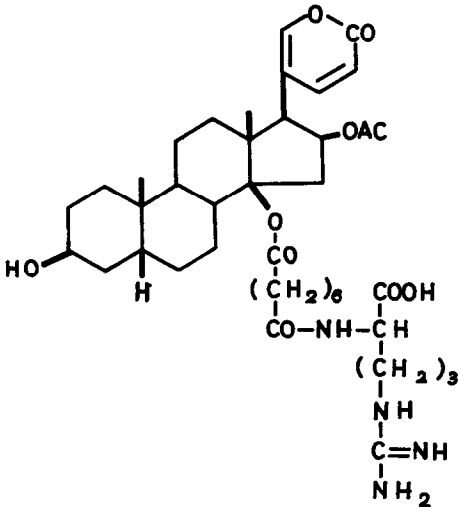
VIII. R = -CO-$(CH_2)_6$-COOH

IX. R = -CO-$(CH_2)_6$-$COOCH_3$



X. bufotoxin

(our suggested formula)



XI. bufotoxin

(= vulgaro bufotoxin)

(H. Wieland's formula)

absorption in IR spectra, II showed a C_3-proton signal at τ 4.84 and exhibited IR absorptions typical to an ester acid. Thus 3-(hydrogen ester) structure was assigned to II. By the treatment with diazomethane, II afforded an methyl ester (III), mp. 105-107°, as colorless prisms from methanol, $C_{35}H_{48}O_9$; λ_{max}^{EtOH} 295 mμ (log ε 3.60); τ 6.32 (3H, s, $COOC\underline{H}_3$). The assigned structure was confirmed by the direct synthesis of cinobufagin 3-(hydrogen suberate) by the reaction of cinobufagin with suberic α-anhydride[6] (mp. 65-66°) in pyridine followed by methylation with diazomethane. The synthetic material was found to be identical with II.

Compound V was obtained as colorless amorphous solid (purity was checked by TLC) and had following spectral properties; λ_{max}^{MeOH} 296 mμ (log ε 3.45); ν_{max}^{KBr} 3500-3100 cm^{-1} (OH), 3040 cm^{-1} (C_{15}-H), 1730-1720 cm^{-1} (lactone and ester CO), 1690 cm^{-1} 1635-1620, 1540 cm^{-1} (C=C of α-pyrone ring), 1240 cm^{-1} (C-O); τ (in $CDCl_3$) 2.12 (1H, dd, J = 10.0 and 2.5 cps, C_{22}-$\underline{H}$), 2.67 (1H, d, J = 2.5 cps, C_{21}-$\underline{H}$), 3.69 (1H, d, J = 10.0 cps, C_{23}-$\underline{H}$), 4.86 (1H, broad peak, C_3-$\underline{H}$), 6.44 (1H, s, C_{15}-$\underline{H}$), 8.99 (3H, s, 19-$C\underline{H}_3$), 9.21 (3H, s, 18-$C\underline{H}_3$). The methyl ester (VI) was derived from V as colorless amorphous solid, and was shown to accord with the formula $C_{33}H_{46}O_7$. Compound V was deduced to be resibufogenin 3-(hydrogen suberate) by comparing with the authentic material, which was synthesized from resibufogenin (IV)[7].

The third compound VIII was isolated in a small amount as amorphous solid. Its methyl ester IX, mp. 154-156°, was obtained as colorless needles, $C_{33}H_{48}O_7$, and had following spectral properties; λ_{max}^{MeOH} 300 mμ (log ε 3.46); ν_{max}^{KBr} 3500 cm^{-1} (OH), 1730-1720 cm^{-1} (lactone and ester CO), 1628, 1535 cm^{-1} (C=C of α-pyrone ring), 1240, 1220, 1200 cm^{-1} (C-O); τ (in $CDCl_3$) 2.06 (1H, dd, J = 9 and 2.5 cps, C_{22}-$\underline{H}$), 2.68 (1H, d, J = 2.5 cps, C_{21}-$\underline{H}$), 3.70 (1H, d, J = 9 cps, C_{23}-$\underline{H}$), 4.86 (1H, broad peak, C_3-$\underline{H}$), 6.33 (3H, s, -$COOC\underline{H}_3$), 9.08 (3H, s, 19-$C\underline{H}_3$), 9.28 (3H, s, 18-$C\underline{H}_3$). These spectral data indicated that VIII was 3-(hydrogen ester) of bufalin (VII)[8]. Thus bufalin 3-(hydrogen suberate) structure was assigned to compound VIII by comparing with the authentic material, which was synthesized from bufalin (VII).

Of the so-called "bufotoxins", Wieland's bufotoxin is the only compound of which structure was extensively studied. Thus Wieland and his co-workers[9] have reported that bufotoxin is 14-suberoylarginine ester of bufotalin (XI). Similar view was expressed by Meyer[10]

and Reichstein[11] although Fieser[12] was suspicious about this conclusion.

Our results, which demonstrated the occurrence of 3-(hydrogen suberoyl)-bufogenins, might be extended to the so-called "bufotoxins" to have suberoylarginine grouping at 3-position (see the formula X). After the completion of the present studies, we learned Meyer[13] had reached the similar conclusion. The present assumption seemed to be reasonable from the standpoint of biogenesis of these classes of compounds. In this connection, reexamination of the structure of "bufotoxins" is also rewarding.

REFERENCES

1. Bufadienolides. II. For previous paper see Y. Kamano, H. Yamamoto, K. Hatayama, Y. Tanaka, M. Shinohara and M. Komatsu, Tetrahedron Letters, in press.
2. Bufogenin 3-(hydrogen suberates) used in this study was extracted from "thin-plate" Ch'an Su, whereas they are also contained in "disk-like" one.
3. M. Komatsu, T. Okano, YAKUGAKU ZASSHI, 87, 712 (1967).
4. H. Linde, P. Hofer, K. Meyer, Helv. Chim. Acta, 49, 1243 (1966).
5. P. Hofer, H. Linde, K. Meyer, Helv. Chim. Acta, 43, 1955 (1960).
6. J. W. Hill, W. H. Carothers, J. Am. Chem. Soc., 55, 5023 (1933).
7. H. Linde, K. Meyer, Helv. Chim. Acta, 42, 807 (1959).
8. M. Kotake, K. Kuwada, Sci. Papers Inst. Phys. Chem. Research, 36, 106 (1939); K. Meyer, Helv. Chim. Acta, 32, 1238 (1949).
9. H. Wieland, R. Alles, Ber., 55, 1789 (1922); H. Wieland, H. Rehringer, Ann., 549, 209 (1941).
10. K. Meyer, Helv. Chim. Acta, 32, 1993 (1949).
11. H. R. Urscheler, Ch. Tamm. T. Reichstein, Helv. Chim. Acta, 38, 883 (1955).
12. L. F. Fieser, M. Fieser, "Steroids", Reinhold Publ. Corp., New York, N. Y., 1959.
13. K. Meyer, Abstracts of Papers, International Symposium on Animal Venoms, Institute Butantan, SÃO PAULO, BRASIL, 1966, p. 58.