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THE ISOLATION AND STRUCTURE OF NEW BUFADIENOLIDE, RESIBUFAGIN AND THE ISOLATION OF MARINOBUFAGIN (1)

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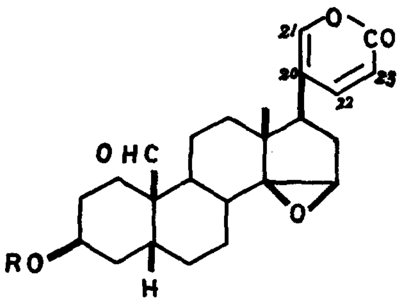
Among some twenty bufadienolides hitherto known, thirteen of them were isolated(2) from the Chinese toad venom drug, Ch'an Su (蟾酥). We have recently described(3) on the detection of unknown compounds from Ch'an Su by using thin-layer chromatography. This report concerns with the isolation and characterization of them.

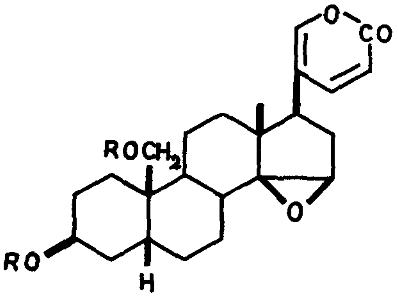
The chloroform extract of Ch'an Su(4) afforded a mixture of unknown materials by column chromatography on silica gel which was eluted by adopting the dry method(5) using an n-hexane-acetone mixture. By rechromatography of the mixture, there were obtained two bufadienolides, one of which was identified to be marinobufagin (V)(6), first isolation from Ch'an Su. The other compound, mp. 210-212°, which was obtained as colorless needles from methanol, was named resibufagin. Based on the following evidence, structure I (3β-hydroxy-19-oxo-14, 15β-epoxy-5β-bufa-20, 22-dienolide) was assigned to the new bufadienolide.

From molecular weight determination (m/e 398) and elemental analysis the compound was found to have the formula $C_{24}H_{30}O_5$. The presence of an α-pyrone ring was indicated from UV (λ^{MeOH}_{max} 301 mμ, logε 3.60) and IR spectra (ν^{KBr}_{max} 1714, 1630, 1535 cm^{-1}). The structure was supported by the NMR spectra ($CDCl_3$), which exhibited signals at τ 2.24 (1H, dd, J = 3 and 10 cps, C_{22}-$\underline{H}$), 2.77 (1H, dd, J = 3 and 1 cps, C_{21}-$\underline{H}$) and 3.79 (1H, dd, J = 10 and 1 cps, C_{23}-$\underline{H}$).(7) The appearance of a signal at a low field of τ 0.50 (1H, s) indicated the presence of a formyl group, the location of which was deduced to be C_{10} based on analogy with



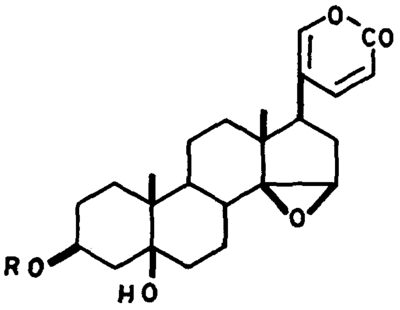
I. R = H resibufagin

II. R = Ac acetyl-resibufagin



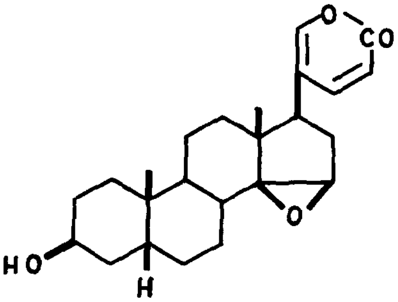
III. R = H resibufaginol

IV. R = Ac acetyl-resibufaginol

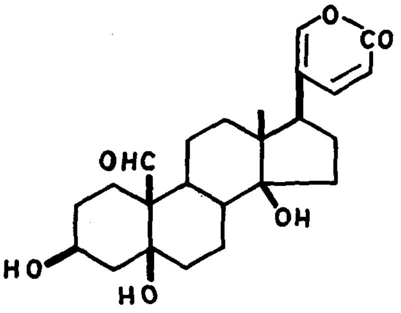


V. R = H marinobufagin

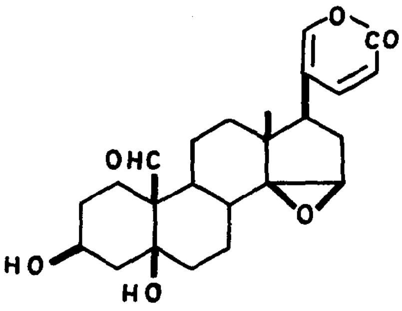
VI. R = Ac acetyl-marinobufagin



VII. resibufogenin



VIII. hellebrigenin



IX. bufotalinin

hellebrigenin, which showed the signal at τ 0.00[7]. 18-Methyl proton showed a singlet at τ 9.14.[7][8][9] A signal at τ 6.49 (1H, s) was assignable to the tertiary proton at C_{15} in 14,15β-epoxy grouping, of which presence was clear from the IR spectral data (3040 cm^{-1}).[8][10] The presence of the epoxy ring was also supported from the IR and NMR spectra of the corresponding acetate (II), mp. 195-199°.

The treatment of I with $NaBH_4$ afforded an alcohol, named resibufaginol (III), mp. 207-210°, which, on acetylation, yielded acetate (IV) as an amorphous solid. Compound III and IV exhibited 19-methylene signal[8] as an AB quartet at τ 6.27 (J = 10.5 cps) and 5.78 (J = 11 cps), respectively.

Marinobufagin (V, 3β, 5-dihydroxy-14, 15β-epoxy-5β-bufa-20, 22-dienolide), mp. 222-224°, was obtained as colorless prisms from acetone. Analytical values and mass spectrum determination (m/e 400) supported the formula $C_{24}H_{32}O_5$. The compound had the following spectral properties; λ_{max}^{MeOH} 300 mμ (logε 3.61); ν_{max}^{KBr} 3400-3000 cm^{-1} (OH), 3040 cm^{-1} (C_{15}-H), 1760, 1640, 1540 cm^{-1}(α-pyrone ring); τ 2.26 (1H, dd, J = 3 and 10 cps, C_{22}-$\underline{H}$), 2.78 (1H, d, J = 3 cps, C_{21}-$\underline{H}$), 3.77 (1H, d, J = 10 cps, C_{23}-$\underline{H}$), 5.83 (1H, broad peak, C_3-$\underline{H}$), 6.48 (1H, s, C_{15}-$\underline{H}$), 9.02 (3H, s, 19-$C\underline{H}_3$), 9.20 (3H, s, 18-$C\underline{H}_3$). 3-Acetate (VI) showed an infrared absorption at 3500 cm^{-1} (C_5-OH) and NMR signals at τ 7.90 (3H, s, $C\underline{H}_3COO$) and 4.76 (1H, broad peak, C_3-$\underline{H}$). These data are consistent with those reported for marinobufagin.[6]

Resibufagin (I) isolated from Ch'an Su is the third bufadienolide having 10-formyl group (the others are hellebrigenin (VIII) and bufotalinin (IX)[11]). Resibufaginol (III) obtained from I in the present studies corresponds to 10-hydroxy compound of resibufogenin (VII), and may be obtained from toad venoms on further examination.[12] It is expected that resibufagin (I) and resibufaginol (III) would show pharmacological activities different from those of resibufogenin (VII).

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12. Until now, hellebrigenol and cinobufaginol were isolated as 19-hydroxy compounds of bufadienolides, and the latter was lately obtained from Ch'an Su by Meyer group (Ref. 8).