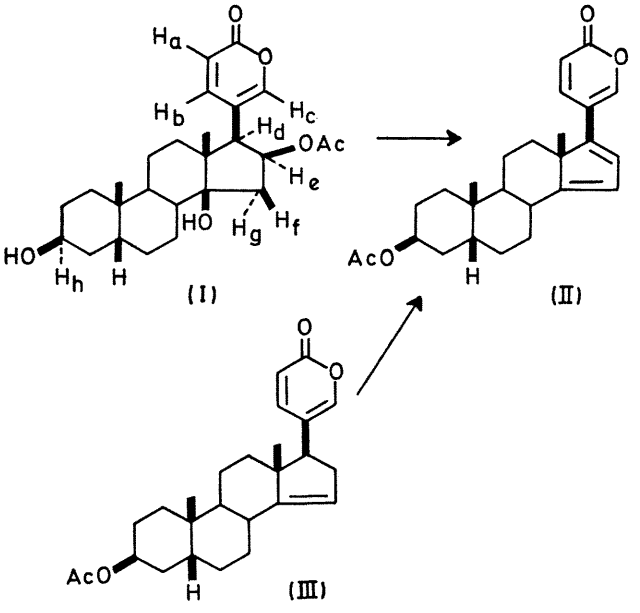
Structure of the Bufadienolide Bufotalin[1]

By GEORGE R. PETTIT,* PETER BROWN, FRED BRUSCHWEILER, and LEONARD E. HOUGHTON
(*Department of Chemistry, Arizona State University, Tempe, Arizona* 85281)

Summary Mass spectral and ^{1}H n.m.r. evidence, coupled with degradation of bufotalin to 3β-acetoxybufotalien (II), in turn prepared by total synthesis, provide unequivocal support for the assignment of structure (I) to bufotalin.

THE structure[2] of bufotalin (I) represents a fundamental milestone in bufadienolide chemistry. A suberylarginine derivative of bufotalin known as bufotoxin is a well known



example of such toad-venom constituents. However, the structures of bufotalin[3] and bufotoxin[4] have been, for good reason, questioned.† We report physical and chemical evidence which places beyond doubt the structure of bufotalin‡ as originally and correctly formulated.

The low-resolution (Atlas CH-4B, molecular-beam inlet system) mass spectrum of bufotalin (I) displayed peaks at the following *m/e* values: 444 (*M*, 5%) (*M* for $C_{26}H_{36}O_6$ = 444); 384 (*M* − 60, 100%); 366 (*M* − 60 − 18, 61%); 351 (*M* − 60 − 18 − 15, 9%); 348 (*M* − 60 − 36, 10%); 341 (*M* − 60 − 43, 80%); 323 (*M* − 60 − 43 − 18, 68%). Metastable-ion peaks at *m/e* 332·0 (440 → 384), 349·0 (384 → 366), and 303·0 (384 → 341) were observed. The presence of one acetoxy-group and two hydroxy-groups attached to the basic steroidal skeleton were definitely indicated.

A ^{1}H n.m.r. (100 MHz) study of bufotalin, employing spin-decoupling experiments, led to the following assignments: the pyrone ring protons, δ 6·17 (H_a, doublet, J_{ab} 9 Hz, J_{ac} 1 Hz), 8·04 (H_b, quartet, J_{ab} 9 Hz, J_{bc} 2·7Hz), and 7·24 (H_c), while in ring D the 17α-proton H_d appeared as a doublet at δ 2·72, J 9 Hz. Because J_{ed} and J_{ef} were both 9 Hz, H_e corresponded to a sextet at δ 5·54 with J_{eg} 2 Hz. The methylene protons H_f and H_g gave signals at δ 2·66 (quartet, J_{ef} 9 Hz and J_{fg} 15 Hz) and 1·9, respectively. The coupling constants noted for the ring-D protons agree well with the stereochemical assignments. A singlet at δ 4·15 (H_h) was diagnostic for the A/B ring stereochemistry. The ^{1}H n.m.r. spectrum of bufotalin was also recorded at 220 MHz, and confirmed the 100 MHz observations.

Conclusive evidence for 5-substitution of the 2-pyrone ring, and overall ring system, was obtained by partial degradation. Bufotalin (I) was converted into 3β-acetoxybufotalien (II) as previously reported.[2] A specimen identical with diene (II) was obtained by heating (6 h) 3β-acetoxy-14-dehydrobufalin (III) with *N*-bromosuccinimide in refluxing carbon tetrachloride. The diene was isolated by column chromatography on silica gel and elution with 1 : 1 benzene–chloroform. Because 14-dehydrobufalin[1,6] has recently been obtained by total synthesis, its conversion into bufotalien constitutes the first total synthesis of this

† Attachment of the suberyl ester group at position-14 of bufotalin also seemed doubtful to us, and in 1959 we (unpublished experiments by V. Thiele and Drs. K. Jaeggi and J. C. Knight) set out to synthesize such a derivative of bufotalin for comparison. While still incomplete, the study did cast doubt on the 14β-hydroxy-group of bufotalin being esterified. Recent results obtained by Kamano and colleagues[5] suggested position-3 as a likely possibility and in a personal communication Professor K. Meyer has informed us that bufotoxin is indeed the 3-conjugate. Meanwhile, we undertook synthesis of the 3-suberylarginine conjugate of bufotalin and a summary of this study is being prepared by G. R. Pettit and Y. Kamano.

‡ An authentic specimen of bufotalin provided by K. Meyer was used to confirm the identity of earlier specimens obtained from a commercial source.

compound and thereby provides compelling support for the bufotalin (I) structural assignment.

This investigation was supported by Public Health Service Research Grants from the National Cancer Institute. We also thank William C. Jankowski, Analytical Instrument division, Varian, for providing the 100 and 220 MHz spectra.

(*Received, September 14th*, 1970; *Com.* 1567.)

[1] For the previous contribution in this series, see G. R. Pettit, L. E. Houghton, J. C. Knight, and F. Bruschweiler, *J. Org. Chem.*, 1970, **35**, 2895.
[2] K. Meyer, *Helv. Chim. Acta*, 1949, **32**, 1993; H. Wieland, J. Hesse, and R. Huttel, *Annalen*, 1936, **524**, 203; H. Kondo and S. Ikawa, *J. Pharm. Soc. Japan*, 1933, **53**, 23 [*Chem. Abs.*, 1934, **27**, 1887].
[3] F. M. Dean, "Naturally Occurring Oxygen Ring Compounds," Butterworth, London, 1963, p. 97.
[4] L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, 1959, p. 792.
[5] Y. Kamano, H. Yamamoto, Y. Tanaka, and M. Komatsu, *Tetrahedron Letters*, 1968, 5673.
[6] G. R. Pettit, L. E. Houghton, J. C. Knight, and F. Bruschweiler, *Chem. Comm.*, 1970, 93.