mediate, possibly the Flory[10] diradical. If so, the stereochemical pattern should be the same; presently available data, not strictly comparable as to temperatures, show order-of-magnitude agreement with a thermal *trans/cis* ratio of 2.2 compared with 3.4 in sensitized photolysis. A further mechanistic correlation accommodates by-products in the 1-phenyltetralin series in photolysis and radiolysis since a 1,6 closure would form the Diels–Alder dimer that, in Mayo's[2] thermal mechanism, is the required precursor. Conversely, in the thermal reaction, this dimer is a possible precursor also in cyclobutane formation since, on bond energy considerations, it is intermediate between styrene and diradical. The still hypothetical Diels–Alder dimer is doubtless a sensitive substance but conceivably could be made in demonstrable amount by low temperature sensitized photolysis of styrene.

Although the radiolysis experiments here described have revealed no products of unequivocally ionic origin, further experimentation should be performed under conditions of intensive drying since, in high polymer formation, this condition is now known[11] to favor ionic mechanisms.

(10) P. J. Flory, *J. Am. Chem. Soc.*, **59**, 241 (1937).
(11) F. W. Burns, R. M. O'Connor, and D. C. Pepper, *J. Polym. Sci., Part B*, **5**, 1059 (1967), and earlier cited literature.

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The Structure of Batrachotoxinin A, a Novel Steroidal Alkaloid from the Colombian Arrow Poison Frog, *Phyllobates aurotaenia*

Sir:

Earlier studies[1,2] on the potent venom from the Columbian arrow poison frog[3] had indicated the presence of three major alkaloids, batrachotoxin, batrachotoxinin A, and batrachotoxinin B. Batrachotoxin, the most active cardiotoxin known (ED_{50}, intravenous in anesthetized dogs,[4] <0.5 μg/kg), was the major steroid alkaloid isolated from these frogs.

When the earlier isolation (distribution between two solvents, preparative tlc) procedures[1,2] were modified and all operations carried out at 5°,[5] very little batrachotoxinin A was present after the initial purification. The major active fraction has now been resolved into two very similar isomers of almost equal toxicity, *viz.*, batrachotoxin (LD_{50}, subcutaneous in mice, 2 μg/kg) and isobatrachotoxin (LD_{50}, 3 μg/kg). In addition, a third component, pseudobatrachotoxin, is present in the extracts and cochromatographs with isobatrachotoxin. It is very unstable and readily converted to batrachotoxinin A, formally by the addition of the elements of water. Thus, batrachotoxinin A is probably a secondary product formed from the highly unstable pseudobatrachotoxin during purification. Batrachotoxinin A (LD_{50}, 1 mg/kg), which retains only $^1/_{500}$ of the toxicity of the original venom, is still almost as toxic as strychnine (LD_{50}, 0.5 mg/kg).[6]

Pure batrachotoxin, isobatrachotoxin, and batrachotoxinin A have now been isolated by column chromatography on silica gel (20W) and by elution with a mixture of cyclohexane, chloroform, triethylamine, and methanol (16:4:1:1). If one assumes that all the batrachotoxinin A arises from pseudobatrachotoxin, then batrachotoxin, isobatrachotoxin, and the sum of pseudobatrachotoxin and batrachotoxinin A occur in fresh extracts in the ratio of 3:1:5. The fragmentation pattern of the high-resolution mass spectrum (AEI MS-9 mass spectrometer, direct inlet 240°) indicates the close relationship of the three isomers ($C_{24}H_{33}NO_4$). Infrared spectra of batrachotoxin[1,2] and isobatrachotoxin show an intense absorption band at 1690 cm^{-1} which is missing in batrachotoxinin A. The band previously reported[2] at 1645 cm^{-1} for batrachotoxinin A is due to an impurity. The ultraviolet spectra of batrachotoxin and isobatrachotoxin are virtually identical and have characteristic maxima at 234 mμ (ϵ 9200) and 264 mμ (ϵ 5100). Batrachotoxinin A and pseudobatrachotoxin have only end absorption as judged from direct measurement and from difference spectra of isobatrachotoxin compared with mixtures of pseudobatrachotoxin and isobatrachotoxin. The previous assumption of only end absorption for batrachotoxin[1,2] rested on the low extinction coefficients now considered to be caused by the presence of pseudobatrachotoxin.

Batrachotoxinin A ($C_{24}H_{35}NO_5$) has now been acylated with *p*-bromobenzoic anhydride under Schotten–Baumann conditions to an O-*p*-bromobenzoate. This product was purified by column chromatography on silica gel (10W) with chloroform containing 3% methanol and recrystallized from acetone to yield fine needles (mp 213°). Mass spectrometry established the composition as $C_{31}H_{38}NO_6Br$. Saponification of this *p*-bromobenzoate with 0.05 *N* alkali at room temperature gave back batrachotoxinin A and not a new or rearranged product.

An X-ray diffraction analysis of a single crystal of this derivative was made using three-dimensional intensity data which were collected using Cu Kα radiation and the equiinclination, multiple-film Weissenberg technique. It was possible to record only a total of 830 independent reflections from a crystal 0.05 × 0.03 mm in cross section. The material crystallizes in the orthorhombic system, space group $P2_12_12_1$ with four molecules in the unit cell and cell dimensions $a = 15.42 \pm 0.03$ Å, $b = 7.05 \pm 0.02$ Å, and $c = 26.50 \pm 0.04$ Å. The Br atom was readily located from a Patterson map to be at 1/5,0,0 and phases based on the position of the Br atom alone gave rise to an electron density map with a fourfold ambiguity for the remainder of the structure. It was possible to identify the two oxygen atoms for the *p*-bromobenzoate group in this map, and thus locate approximately the *p*-bromobenzoate group in the unit cell. Using this group as a known partial structure, phases were obtained for some of the strongest reflections. These phases were used with the tangent formula in recycling procedure[7] to

(1) F. Märki and B. Witkop, *Experientia*, **19**, 239 (1963).
(2) J. W. Daly, B. Witkop, P. Bommer, and K. Biemann, *J. Am. Chem. Soc.*, **87**, 124 (1965).
(3) Previously referred to as *Phyllobates bicolor;* see footnote 22 in J. W. Daly and C. W. Myers, *Science*, **156**, 970 (1967).
(4) L. S. Harris and F. J. Rosenberg, personal communication. This dose causes interference with conduction in the heart and produces extra systoles. Slightly higher doses cause ventricular fibrillation and death. Little effect on blood pressure was noted.
(5) J. W. Daly and B. Witkop, *Mem. Inst. Butantan, Suppl. Comemorative*, 2, in press.
(6) *Cf.* N. P. Christy, *Am. J. Med.*, **42**, 111 (1967).
(7) J. Karle, *Acta Cryst.*, **21**, in press.

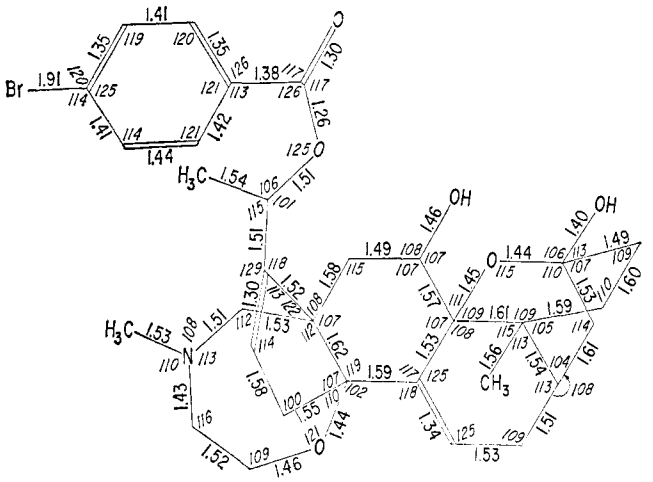
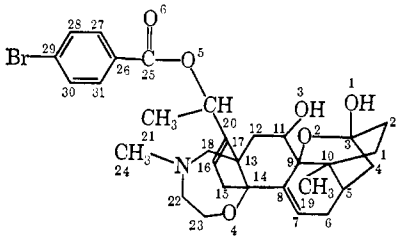


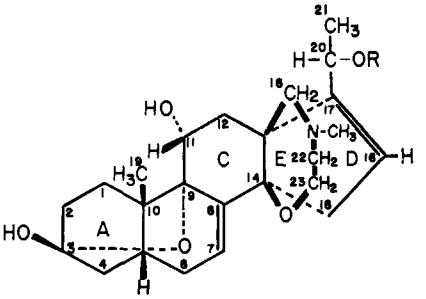
Figure 1. Bond lengths and angles of batrachotoxinin A *p*-bromobenzoate (I).

obtain additional phases. Further atoms were found in the resulting E maps. In several cycles all 39 atoms were located. E maps computed from phases obtained in this manner are much better resolved than those obtained by the heavy atom method. In this particular case, the fact that the heavy atom was specially placed and the data were severely limited made the direct application of the heavy atom method quite unsuitable.

The identification of the six oxygen atoms was obvious from the weights of the peaks in the E maps. The identification of the nitrogen atom was based on the size of the thermal factors in the least-squares refinement, difference maps, and bond lengths. A least-squares refinement of the coordinates for each atom, isotropic thermal factors for all atoms except for the Br atom, and anisotropic thermal factors for the Br atom resulted in an agreement factor of 9.7%. In this way structure I was obtained with the bond lengths and angles shown in Figure 1.



I



II, R = H; BrC_6H_4CO-

Structure I, when expressed as a steroid derivative, becomes 3α,9α-epoxy-14β,18β-[epoxyethano-N-methyl-imino]-5β-pregna-7,16-diene-3β,11α,20α-triol (II) which is the structure of batrachotoxinin A. The three-dimensional picture of this novel steroid is presented in the stereodrawing, Figure 2. The structural relationship and the pharmacology of batrachotoxin, isobatrachotoxin, pseudobatrachotoxin, and batrachotoxinin A will be the subject of future papers.

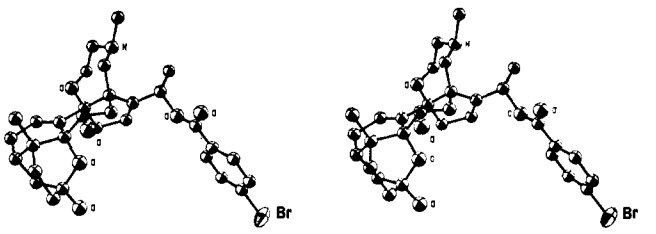


Figure 2. Stereodrawing of the configuration of batrachotoxinin A as determined by X-ray analysis. The drawing was made by a computer from a program prepared by C. Johnson, Oak Ridge National Laboratory. The picture should be seen with a three-dimensional viewer for printed stereophotographs (commercially available, *e.g.*, from Stereo-Magniscope, Inc., Elmhurst, N. Y.).

(8) Associate in the Visiting Program of the USPHS, 1965–1968.

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Ultraviolet Spectrum of the ClOO Radical

Sir:

The ClOO radical was first proposed by Porter and Wright[1] as an intermediate in the flash photolysis of chlorine–oxygen mixtures.

$$Cl_2 + h\nu \longrightarrow Cl + Cl$$
$$Cl + O_2 + M \rightleftharpoons ClOO + M$$
$$Cl + ClOO \longrightarrow ClO + ClO$$
$$Cl + ClOO \longrightarrow Cl_2 + O_2$$
$$ClO + ClO \longrightarrow Cl_2 + O_2$$

From absorption spectroscopy in the ultraviolet (2600–3000 Å), they observed the intermediate diatomic radical ClO. Although not observed, the peroxy radical was postulated to be a short-lived precursor to ClO in their system. Since then, the ultraviolet absorption spectrum of ClO has been observed repeatedly;[2] however, until very recently no spectrographic evidence existed for the presence of ClOO. Rochkind and Pimentel[3a] detected a new infrared absorption in a matrix-isolation study, and Arkell and Schwager[3b] have shown this to be one band in the infrared spectrum of ClOO.

The molecular modulation apparatus described elsewhere[4] has been adapted for work in the ultraviolet.

(1) G. Porter and F. J. Wright, *Discussions Faraday Soc.*, **14**, 23 (1953).
(2) M. A. A. Clyne and J. A. Coxon, *Trans. Faraday Soc.*, **62**, 1175 (1966).
(3) (a) M. M. Rochkind and G. C. Pimentel, *J. Chem. Phys.*, **46**, 4481 (1967); (b) A. Arkell and I. Schwager, *J. Am. Chem. Soc.*, **89**, 5999 (1967).
(4) H. S. Johnston, G. E. McGraw, T. T. Paukert, L. W. Richards, and J. Van den Bogaerde, *Proc. Natl. Acad. Sci. U. S.*, **57**, 1146 (1967).