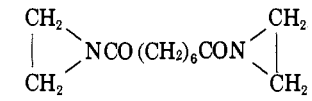

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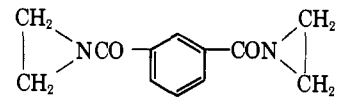

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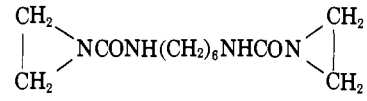

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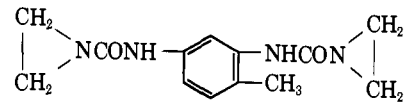

IV

dinecarboxamide (Table I) has been found to produce the same effect at 0.01% concentration. Although other comparisons with difunctional compounds in Table I are not available, it appears that in the diaziridinyl series the acyl–carbamoyl exchange is highly significant.

### Experimental

**General Method for Carbamoylaziridines.**—A solution or suspension containing 0.1 equiv. of the isocyanate in 100 ml. of solvent (ether or benzene) was added over a 10-min. period to 0.2 equiv. of the aziridine in 100 ml. of solvent. The reaction mixture was cooled in an ice bath during the addition and then kept at room temperature for 1 hr. The carbamoylaziridine usually started to crystallize at this point, and the reaction mixture was cooled for 1 hr. in an ice bath. In a few instances it was necessary to remove most of the solvent before crystallization occurred. The products were collected by filtration and recrystallized from anhydrous ether or benzene.

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## Cytotoxicity of Cardiac Principles

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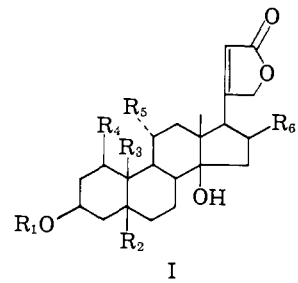
Cytotoxicity associated with cardiac principles and certain other steroidal derivatives has been reported in recent publications.[1,2] We are prompted by these reports to publish some of our observations in this respect.

During an investigation of the cytotoxicity of extracts of the bulbs of *Ornithogalum umbellatum*, we isolated an active principle as a crystalline compound and identified it as convallatoxin. This cardiac glycoside is a potent cytotoxic agent having an $ID_{50}$ of 0.002 $\gamma$/ml. when assayed against Eagle's KB strain of human epidermoid carcinoma.[3] It also showed a correspondingly high activity (8000 Bu./mg.) in a disk-plate assay against KB cells in agar.[4]

The discovery of the cytotoxicity of convallatoxin led us to test some available cardiac aglycones and glycosides for the same activity. The results, presented in Table I, demonstrate that all of the compounds tested had significant activity and most of them had activity of a high order.

The data presented in Table I, in conjunction with that available in the literature,[1,2] suggest that cytotoxicity is associated with an unsaturated lactone either attached to position 17 by a carbon–carbon bond or fused to ring D across the 16,17-position. Thus, the cardiac principles have either a cardenolide ring (as in I) or a bufadienolide ring (as in II) attached to position 17, while the most active of 150 steroids tested in these laboratories have an $\alpha,\beta$-unsaturated $\gamma$-lactone fused to the 16,17-positions.[1] The data in Table I also indicate that, in our assay, the glycosides are more active than the corresponding aglycones and that the cardenolide and bufadienolide rings are equally effective in conferring activity. The two most potent compounds in Table I, convallatoxin and hellebrin, have the following structural features in common: (a) they are both rhamnosides, (b) they both have $\beta$-hydroxyl groups at positions 5 and 14, and (c) they both have the C-19 carbon atom represented by an aldehyde function. However, the former has a cardenolide ring at position 17 while the latter has a bufadienolide ring at the same position.



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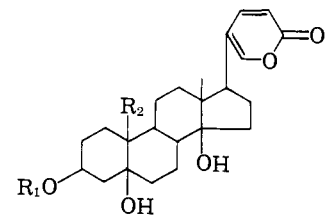

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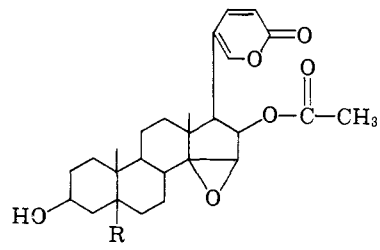

III

### Experimental

Cytotoxicity of the extracts and purified fractions is determined by a disk-plate assay against KB cells in agar.[4]

**Isolation of Convallatoxin.**—*Ornithogalum umbellatum* bulbs (27 kg., air dried at room temperature) were ground and stirred in 130 l. of water at 85° for 1 hr. The solids were removed by centrifugation and re-extracted as above with 95 l. of water. The aqueous extracts were extracted in a continuous extractor with 1.5 vol. of 1-butanol. The butanol extracts were evaporated *in vacuo* to a dark brown tar weighing 270 g. and assaying 56 Bu./mg. A portion of the tar (87 g., 4.9 × $10^6$ Bu.) was subjected to a 25-transfer countercurrent distribution between the two phases of

(1) J. E. Pike, J. E. Grady, J. S. Evans, and C. G. Smith, *J. Med. Chem.*, **7**, 348 (1964).

(2) (a) S. M. Kupchan, R. J. Hemingway, and R. W. Doskotch, *ibid.*, **7**, 803 (1964); (b) S. M. Kupchan, J. R. Knox, J. E. Kelsey, and J. A. S. Renauld, *Science*, **146**, 1685 (1964).

(3) For details of this assay see C. G. Smith, W. L. Lummis, and J. E. Grady, *Cancer Res.*, **19**, 843 (1959).

(4) This assay was a modification of Miyamura's technique [S. Miyamura, *Antibiot. Chemotherapy*, **6**, 280 (1956)]; details are to be published later. Bu means biological units.

TABLE I
CYTOTOXICITY OF CARDIAC PRINCIPLES

| Compd. | Structure | Disk-plate assay,[a] Ru./mg. | Tube-dilution assay,[b] $LD_{50}$, γ/ml. |
|---|---|---|---|
| Strophanthidin | I, $R_1 = R_4 = R_5 = R_6 = H$; $R_2 = OH$; $R_3 = CHO$ | 140 | 0.11 |
| Convallatoxin | I, $R_1$ = L-rhamnose; $R_2 = OH$; $R_3 = CHO$; $R_4 = R_5 = R_6 = H$ | 8000 | 0.002 |
| K-Strophanthin-β | I, $R_1$ = glucose–cymarose; $R_2 = OH$; $R_3 = CHO$; $R_4 = R_5 = R_6 = H$ | 13000 | 0.023 |
| Ouabain | I, $R_1$ = L-rhamnose; $R_2 = R_4 = R_5 = OH$; $R_3 = CH_2OH$; $R_6 = H$ | 13000 | 0.024 |
| Digitoxigenin | I, $R_1 = R_2 = R_4 = R_5 = R_6 = H$; $R_3 = CH_3$ | 400 | 0.078 |
| Digitoxigenin 3-acetate | I, $R_1 = CH_3CO$; $R_2 = R_4 = R_5 = R_6 = H$; $R_3 = CH_3$ | 130 | 0.11 |
| Gitoxigenin | I, $R_1 = R_2 = R_4 = R_5 = H$; $R_3 = CH_3$; $R_6 = OH$ | 411 | 0.58 |
| Oleandrigenin | I, $R_1 = R_2 = R_4 = R_5 = H$; $R_3 = CH_3$; $R_6 = CH_3CO_2$ | 460 | 0.059 |
| Oleandrin | I, $R_1$ = L-oleandrose; $R_2 = R_4 = R_5 = H$; $R_3 = CH_3$; $R_6 = CH_3CO_2$ | 900 | 0.013 |
| Telocinobufagin | II, $R_1 = H$; $R_2 = CH_3$ | 800 | 0.02 |
| Hellebrin | II, $R_1$ = glucose–L-rhamnose; $R_2$ = CHO | 8000 | 0.006 |
| Cinobufagin | III, R = H | 800 | 0.028 |
| Cinobufotalin | III, R = OH | 800 | 0.063 |

[a] See ref. 4. [b] See ref. 3.

the solvent system: water–ethanol–1-butanol–Skellysolve B (10:2:8:5); the volume of each phase was 50 ml. After distribution, the contents of tubes 5–18, containing most of the activity, were combined and evaporated *in vacuo*. The residue (13.3 g.) was dissolved in 20 ml. of ethylene dichloride–methanol (1:1) and applied to a column of 350 g. of silicic acid. The column was eluted in 25-ml. fractions with 1250 ml. each of ethylene dichloride–methanol (9:1) and ethylene dichloride–methanol (3:1). Fractions 41–89, which contained most of the activity were combined and evaporated to dryness *in vacuo*. The residue ($3.9 \times 10^6$ Bu.) was subjected to a 600-transfer countercurrent distribution between the two phases of the solvent system: chloroform–acetone–water (1:2:2); the volume of each phase was 10 ml. After distribution the contents of tubes 411–460, which contained the activity, were combined and evaporated *in vacuo* almost to dryness. On cooling, the concentrate deposited crystalline material which, on crystallization from acetone–water, yielded 452 mg. of convallatoxin, m.p. 228–231°, identical with an authentic sample of convallatoxin (melting point, mixture melting point, ultraviolet, infrared, and n.m.r. spectra).

*Anal.* Calcd. for $C_{29}H_{42}O_{10}$: C, 63.25; H, 7.69. Found: C, 62.95; H, 7.94.

**Acknowledgment.**—We are indebted to Dr. S. P. Owen and M. R. Burch for biological assays and to Dr. George Slomp and associates for analysis and determination of spectra. The authors thank Dr. J. W. Hinman, Head of our Natural Products Section, for his interest and advice.

## The Relationship between Structure and Anticonvulsant Activity in a Series of Benzenesulfonamides

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During the course of routine testing, it was noted that 4-bromobenzenesulfonamide exerted a potent anticonvulsant effect as measured by protection against maximal electroshock (ES) seizures, thiosemicarbazide (TSC) lethality, or strychnine (Strych.) lethality. The availability of many closely related compounds suggested a unique opportunity to explore the relationship between chemical structure and activity. The present report details the synthesis of several new sulfonamides and the pharmacological study of the entire series.

### Results and Discussion

The $LD_{50}$ and anticonvulsant data obtained in these studies are detailed in Table I. Examination of the data on effects of 4-substitution (compounds **1**–**16**) reveals a wide variation between anticonvulsant activity and lethality. The most toxic compound (**13**) is devoid of anticonvulsant activity. The four most active compounds (**3**–**6**) are active in all three test procedures. The TSC and Strych. tests do not discriminate among these four agents; however, ES reveals the order $CF_3 > Br = Cl > I$. Further, it is clear that, in terms of ratio of activity to lethality, the 4-bromo compound is the most effective agent. Subsequent studies of the effect of variation in substitution on the sulfonamide N were carried out utilizing the 4-Br substitution.

The study of N-substituted compounds (**17**–**34**) reveals that introduction of simple monoalkyl substituents does not abolish activity but, in general, increases toxicity. When the substituent is *n*-butyl or larger (**25**–**30**), activity is generally lost. With the exception of dimethyl (**31**), the disubstituted compounds (**32**–**34**) are inactive in the anticonvulsant tests. This pattern of activity suggested that the activity of these compounds might be the result of biotransformation. Studies designed to clarify this hypothesis are reported by Smith, *et al.*, in a subsequent manuscript.

#### Experimental

**Methods.**—All experiments were carried out in Carworth Farm male mice weighing 20–25 g. Toxicity was determined