and evaporated on a steam bath. The residue was fractionated under reduced pressure. The bases gave crystalline salts with difficulty; those which were obtained are described in Table I, after the respective base.

Pharmacology.—For all compounds, we evaluated (using codeine as reference substance) the antitussive activity on cats,[5,6] observing whether this was accompanied by hypotension and respiratory impairment, the acute LD_{50} in mice and albino rats,[7] and the local anesthetic[8] and analgesic activity.[9,10] The occurence of central sedation was measured through variations in the spontaneous motility and in the performance of the grip test, and through the influence exerted by the compounds on the duration of the barbiturate narcosis.[11] The spasmolytic activity was tested on the isolated rat and guinea pig ileum against histamine, acetylcholine, $BaCl_2$, and nicotine contractions.[12]

Compound **15** was studied as antitussive also on unanesthetized rats and guinea pigs[13,14] and further as an inhibitor of pentylenetetrazole seizures,[11] for its effect on peristalsis,[15] and on the bronchial and tracheal muscles *in vivo* and *in vitro*[16,17]; finally its influence upon the bronchial and tracheal secretion was examined.[18]

In Table III only the compounds appreciably active as antitussives are recorded with the results concerning their antitussive, local anesthetic, analgetic, narcosis-enhancing, hypotensive, and respiratory-depressing activities and the data about their acute toxicity.

A few observations relating the structure to the pharmacological activities are to be made. The antitussive as well as the other pharmacological activities listed are generally more pronounced in the thymol than in the guaiacol derivatives. The antitussive effect lasted for a time roughly proportional to the activity index (from 30 min. to 3–4 hr.) and was sometimes accompanied by a depression of the respiratory function. The compounds of both series containing a diethyl radical were inactive, whatever the length of the chain. In agreement with the literature,[19] the piperidine group seems to enhance this activity, as clearly shown by **15** (though a guaiacol derivative) and **18**, both being the most active of all our antitussives.

As spasmolytics the guaiacol derivatives were completely inactive, while the thymol derivatives were very weak inhibitors of acetylcholine contractions (less than 1% of atropine), and of about the same activity as papaverine against $BaCl_2$ contractions and hexamethonium against nicotine spasms.

The behavioral pattern,[20] body temperature, motility, and muscular tonus and force of the treated animals as well as the response to the hot plate test for analgesia were unaffected; the only signs (more evident in the thymol derivatives) of a certain trend to affect CNS functions were the analgesia detected in the phenylquinone test and the moderate prolongation of the barbiturate narcosis. These mild and unspecific effects on the CNS are in contrast with the marked sedative properties of some thymol derivatives described in literature.[2] Therefore, the observed antitussive effect does not seem to be bound to general analgesia or sedation, or to the intensity of the local anesthetic effect; in fact for some active compounds hypotensive and respiratory-depressing effects were lacking.

Compound **15** was studied particularly since it was the only one which showed a good degree of activity also by the oral route. Its antitussive activity is of same order of magnitude as that of codeine in the experiments on cats and seems to be fairly specific because of lack of side effects (respiratory and circulatory implications, inhibition of intestinal peristalsis, bronchoconstriction, and general sedation). No protection against pentylenetetrazole seizures and no change in the mucous tracheal secretion were observed. It did not produce tolerance; in cats injected daily for 4 weeks with 10 mg./kg. i.p., the inhibitory effect on the experimental cough[5,6] was obtained by the same doses which were active in cats not pretreated.

It does not appear that the hypotensive activity shown by several compounds is cause for concern. It is presumably bound to myocardic impairment as seen on the isolated rabbit heart, and to an intense and persistent peripheral vasodilatory effect, as also seen in the isolated rabbit ear vessels,[21] together with an adrenolytic effect; in any event, this was not observed on the blood pressure response to epinephrine.

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Insect Chemosterilants. II.[1] N-Carbamoylaziridines

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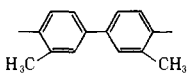
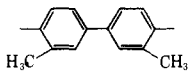
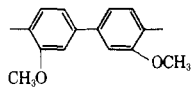
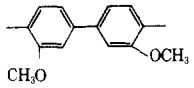
Certain N-substituted aziridines are outstanding in their ability to interfere with insect reproduction. This type of biological activity can be found among other alkylating agents (*e.g.*, nitrogen mustards, alkyl alkanesulfonates), but the aziridines proved to be particularly useful because of their effectiveness in sterilizing male insects and their often wide toxicity–sterility margin.[2]

In general, the aziridine bases with no substituent on the ring nitrogen and the N-alkylaziridines have little or no activity as insect sterilants. Most of the screening and synthesis of aziridinyl chemosterilants centered on the compounds which had an unsaturated group attached to the ring nitrogen. The N-acyl and N-aroylaziridines exhibited the sterilizing activity in various degrees, but none of the compounds tested approached the high potency of tepa or other phosphorus-containing aziridines.[3]

It appeared desirable to increase the electron deficiency of the carbonyl carbon of N-acylaziridines by replacing the acyl group with a carbamoyl group. Several diaziridinyl compounds of this type were commercially available, and their testing and comparison with similar N-acylaziridines indicated substantially increased sterilizing properties. In our previous communication,[1] we have reported the synthesis of 1,1′-suberoylbisaziridine (I) and 1,1′-isophthaloylbisaziridine (II). The first compound exhibited no appreciable sterilizing properties when fed to house flies (*Musca domestica* L.) at 1% concentration and the second produced a 75% reduction in egg hatch when fed at 5% concentration. On the other hand, the two similar compounds N,N′-hexamethylenebis-1-aziridine-

(1) Part I in the series: C. W. Woods, A. B. Bořkovec, and F. M. Hart, *J. Med. Chem.*, **7**, 371 (1964).
(2) A. B. Bořkovec, *Residue Rev.*, **6**, 87 (1964).
(3) S. C. Chang and A. B. Bořkovec, *J. Econ. Entomol.*, **57**, 488 (1964).

TABLE I

R_1	R_2	B.p. (mm.) or m.p., °C.[a]	Yield, %	% Nitrogen Calcd.	% Nitrogen Found	Sterilizing activity,[b] % hatch
Monofunctional N-Carbamoylaziridines						
$CH_3(CH_2)_3$	H	85–87 (0.4)	74	19.70	19.82	Normal
$CH_3(CH_2)_3$	CH_3	86–87 (0.2)	69	17.94	17.86	Normal
o-$CH_3C_6H_4$	H	75–76	78	15.90	15.74	6
m-$CH_3C_6H_4$	H	70.5–71.5	65	15.90	15.65	17
p-$CH_3C_6H_4$	H	98.5–99.5	70	15.90	15.67	8
o-$CH_3OC_6H_4$	H	64–65	73	14.58	14.63	Normal
p-$CH_3OC_6H_4$	H	114–115.5	93	14.58	14.40	Normal
o-ClC_6H_4	H	52–53.5	57	14.25	14.33	Normal
m-ClC_6H_4	H	92.5–94	82	14.25	14.15	Normal
p-ClC_6H_4	H	132–133.5	65	14.25	14.36	Normal
p-$NO_2C_6H_4$	H	164 dec.	92	20.28	20.09	Normal
1-Naphthyl	H	108–109	56	13.20	13.08	Normal
1-Naphthyl	CH_3	116–117	68	12.38	12.24	Normal
Difunctional N-Carbamoylaziridines						
CH:CH (*trans*)	H	260 dec.	90	28.56	28.31	0
	H	210 dec.[c]	88	18.91	18.98	0
	CH_3	205 dec.	80	17.27	17.22	0
	H	330 dec.	86	15.99	15.51	Normal
	CH_3	315 dec.	93	14.80	14.57	Normal
	H	173	56	14.56	14.66	59
	CH_3	115–118	26	13.65	13.66	Normal

[a] All melting points are corrected. [b] Compound fed at 1% concentration for 3 days to freshly emerged flies of both sexes; untreated flies gave 85–100% (normal) hatch. [c] Cytotoxic properties of this compound were reported by J. A. Hendry, R. F. Homer, F. L. Rose, and A. L. Walpole, *Brit. J. Pharmacol.*, **6**, 357 (1951), but the mode of preparation and physical characteristics were not given.

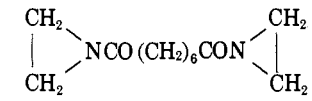
carboxamide (III) and N,N′-(4-methyl-*m*-phenylene)-bis-1-aziridinecarboxamide (IV)[4] proved to be rather potent house fly sterilants which completely eliminated the egg hatch at 0.1% concentration.

We wish to report the synthesis of twenty N-carbamoylaziridines which were prepared as candidate chemosterilants. All of these compounds were synthesized from ethylenimine or 2-methylaziridine and commercially available isocyanates; their physical characteristics are summarized in Table I. The last column in Table I contains preliminary house fly screening data[5] obtained by Dr. G. C. LaBrecque and his associates in the Entomology Research Division, U.S.D.A., in Gainesville, Fla.

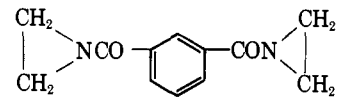
Among the monofunctional carbamoylaziridines, only the tolyl compounds showed a significant sterilizing activity, whereas the other monoaziridines were only slightly or not at all active. Because the corresponding N-acylaziridines had only slightly lower or equal activity (or were equally inactive), it can be concluded that the effect of the replacement of an acyl with a carbamoyl group is insignificant in the monoaziridinyl series. In the diaziridinyl series two pairs of compounds were already mentioned (I and III, and II and IV); another pair is now available. The previously reported[4] 1,1′-fumaroylbisaziridine completely inhibited the egg hatch at 0.5% concentration, but the corresponding N,N′-*trans*-vinylenebis-1-aziri-

(4) Boric compounds were obtained from Chemirad Corp., East Brunswick, N. J.

(5) For detailed procedure of screening house fly chemosterilants see G. C. LaBrecque, *J. Econ. Entomol.*, **54**, 684 (1961).



I



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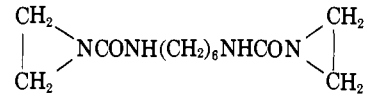
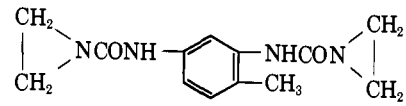


III



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.

Cytotoxicity of Cardiac Principles

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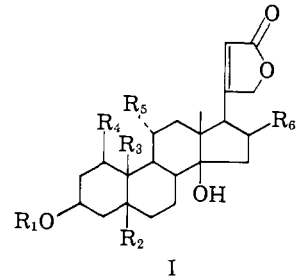
Cytotoxicity associated with cardiac principles and certain other steroidal derivatives has been reported in recent publications.[1,2] We are prompted by these reports to publish some of our observations in this respect.

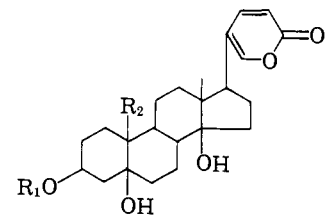
During an investigation of the cytotoxicity of extracts of the bulbs of *Ornithogalum umbellatum*, we isolated an active principle as a crystalline compound and identified it as convallatoxin. This cardiac glycoside is a potent cytotoxic agent having an ID_{50} of 0.002 γ/ml. when assayed against Eagle's 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.

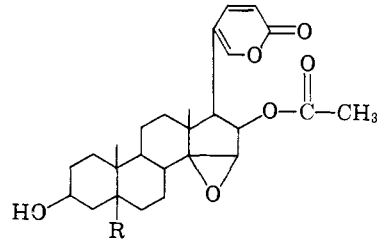
The data presented in Table I, in conjunction with that available in the literature,[1,2] suggest that cytotoxicity is associated with an unsaturated lactone either attached to position 17 by a carbon–carbon bond or fused to ring D across the 16,17-position. Thus, the cardiac principles have either a 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 α,β-unsaturated γ-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 β-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.



I



II



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.