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STUDIES ON SOLANUM ALKALOIDS: II. THE ANTI-ACCELERATOR CARDIAC ACTION OF SOLASODINE AND SOME OF ITS DERIVATIVES * †

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In a previous communication (Krayer and Briggs, 1950) it was shown that the solanum alkaloids β-dihydrosolasodine and tetrahydrosolasodine have an antagonistic action to the positive chronotropic effect of adrenaline on the mammalian heart which is similar to that of the veratrum alkaloids veratramine and jervine. For chemical reasons, which will be considered in detail elsewhere, it is preferable to adhere as strictly as possible, with our limited knowledge of configuration so far, to a more systematic nomenclature than that adopted previously. Hence, in the present communication β-dihydrosolasodine will be called dihydrosolasodenol and tetrahydrosolasodine will be named dihydrosolasodanol.

Using the heart-lung preparation of the dog, we have examined solasodine itself and its hydrogenation product solasodanol, as well as two of its naturally occurring glycosides, solasonine and solmargine, and have carried out further experiments with dihydrosolasodenol and dihydrosolasodanol in an attempt to make a quantitative comparison of all these compounds. The closely related tertiary amine solanidine was included in this study in order to see whether the anti-accelerator potency is a property of the secondary amines only, as experiments with cevine appeared to indicate for the veratrum alkaloids.

Further chemical evidence and reconsideration of its existing properties, the details of which will be published elsewhere, support the revised formula (I) for solasonine and (II) for solasodine (Briggs *et al.*, 1950). The position of one attachment of the oxide linkage to C_{16} is still uncertain but is placed in this position on analogy with the saponins of which it may be regarded as the part nitrogen analogue. In respect to its being a secondary amine solasodine now resembles solanocapsine from *Solanum pseudocapsicum* (Barger and Fraenkel-Conrat, 1936), the veratrum alkaloids jervine (Jacobs and Sato, 1948), veratramine (Jacobs and Craig, 1945), and an unnamed base, $C_{27}H_{41(39)}O_4N$, from *Veratrum viride*, Aiton (Jacobs and

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Craig, 1945). Jacobs and Huebner (1947) have suggested several structures for jervine, including one containing the same type of spirodiheterocyclic system.

On the above formulation of solasodine (II) solasodanol (Briggs, Newbold, and Stace, 1942) may now be regarded as (III) and dihydrosolasodenol, a new compound (Briggs *et al.*, 1950), obtained by one of us (L. H. Briggs) and R. H. Locker by the action of lithium aluminum hydride on solasodine, as (IV). Dihydrosolasodenol is converted to dihydrosolasodanol (V) by catalytic hydrogenation with a palladium catalyst.

The solasonine used in this study occurs in *Solanum aviculare* (Bell and Briggs, 1942), a plant endemic to Australia and New Zealand, in *Solanum sodomeum* (Oddo and Colombano, 1905), native to the countries bordering the Mediterranean Sea but now also growing wild in New Zealand, and in *Solanum xanthocarpum* (Saiyed and Kanga, 1936) and *Solanum torvum* (Krishnamurti and Seshadri, 1949), indigenous to India.

The solanidine was prepared from *Solanum tuberosum*. Its structure and tertiary amine nature (VI) has been established by the work of several authors (Rochelmeyer, 1938; Craig and Jacobs, 1943; Prelog and Szpilfogel, 1944; Uhle and Jacobs, 1945). Professor Prelog, of the Department of Chemistry, Eidg. Technische Hochschule, Zürich, generously supplied us with another sample of solanidine prepared by him. Solanine has been shown (Briggs *et al.*, 1950) to have its trisaccharide moiety similarly constituted to that of solasonine.

Solmargine is a new alkaloid from *Solanum marginatum*, which has been shown (Briggs *et al.*, 1950) to be a trisaccharide derivative of solasodine, the sugars being rhamnose, glucose, and a third component, still uncertain, probably rhamnose.

Some chemical and physical data concerning the substances used in this study are given in Table I.

TABLE I

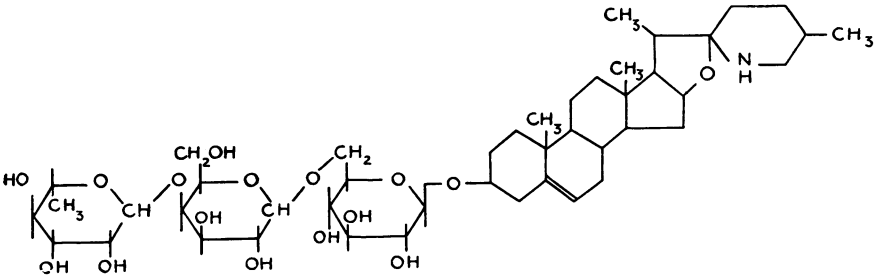
CHEMICAL AND PHYSICAL PROPERTIES OF SEVERAL SOLANUM ALKALOIDS

Substance	Empirical formula	Mol. wt.	Melt. point. ° C.	No. of exps.
Solasodine	$C_{27}H_{43}NO_2$	413.62	201–202	2
Solasodanol	$C_{27}H_{45}NO_2$	415.64	207–208.5	3
Dihydrosolasodenol	$C_{27}H_{45}NO_2$	415.64	260–264	3
Dihydrosolasodanol	$C_{27}H_{47}NO_2$	417.65	290–295	5
Solasonine	$C_{45}H_{73}NO_{16}$	884.04	285–287	1
Solmargine	$C_{44}H_{71}NO_{15}$	854.02	280–289*	2
Solanidine	$C_{27}H_{43}NO$	397.62	218–219	2

* On further purification the melting point of Solmargine rose to 300–301° C.

METHODS

This study is based on the results of 18 experiments on the heart-lung preparation of the dog (HLP). The animals had an average weight of 10.4 kg. (7.3–14.6), 15 were male and 3 female. Dose-response curves were obtained as described elsewhere (Krayer, 1950);



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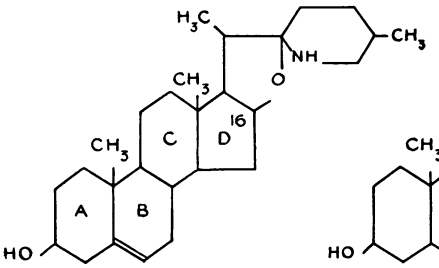

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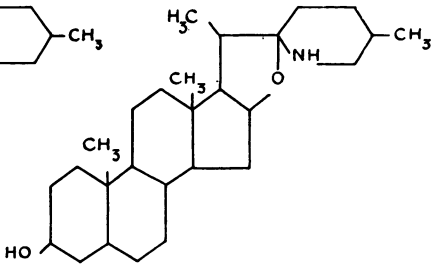

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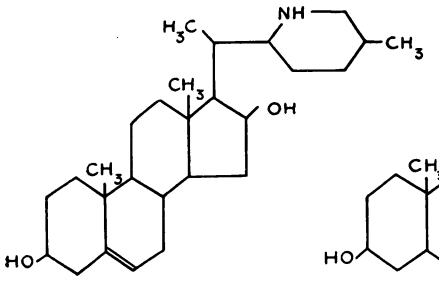

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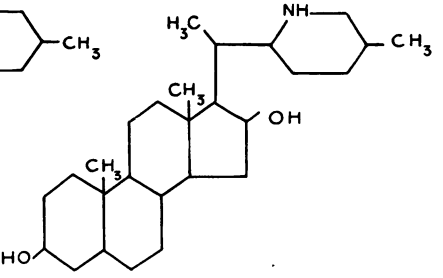

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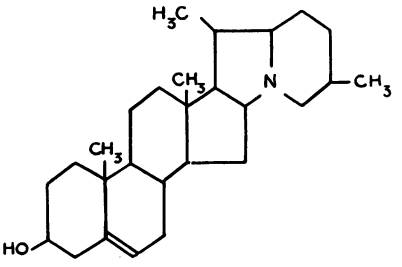

VI

3 μg. *l*-adrenaline* base per min. were given by continuous infusion to maintain a steady state of cardio-acceleration, and at intervals suitable doses of an anti-accelerator substance were added to obtain appropriate increments of inhibition. From such experiments the dose causing 50 per cent inhibition (I_{50} value) could be derived. The total number of experiments made with each individual substance is given in Table I. With the exception of dihydrosolasodanol this number was determined by the limited amount of each substance available for the study. (For further details of the experimental procedure see Krayer, 1950.)

The solanum alkaloids were brought into solution as hydrochlorides using approximately 0.25 c.c. 0.1 N-hydrochloric acid (a little more than the equivalent amount) for every 10 mg. of the alkamines, or, with solmargine and solasonine, for every 10 mg. of the genins.

The isotonic solutions for administration to the heart-lung preparation were prepared with 5 per cent (w/v) dextrose solution. The concentration of solasodine and solasodanol was 1:4,000; of dihydrosolasodanol 1:2,000 or 1:4,000; of dihydrosolasodenol and solanidine 1:2,000. The more readily soluble salts of solasonine and solmargine were used in a concentration of 1:100 and 1:10 respectively of the glycosides. All doses mentioned refer to the alkaloids unless otherwise indicated.

Atropine was given in some of the experiments; all doses refer to atropine sulphate.

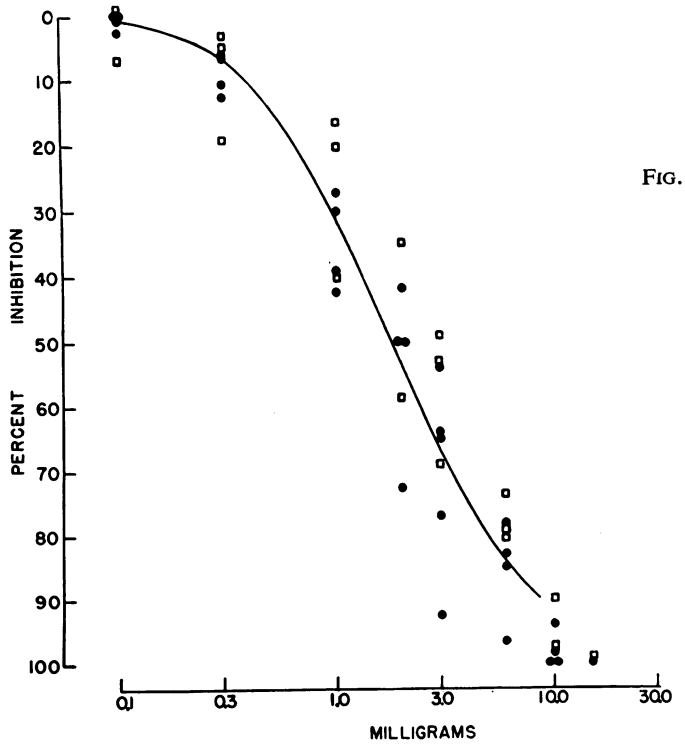

FIG. 1.—The anti-accelerator activity of dihydrosolasodenol and dihydrosolasodanol. Heart-lung preparation of the dog. Eight experiments. Cardio-acceleration was produced by continuous infusion of 3 micrograms of *l*-adrenaline base per minute. Ordinate: percentage inhibition of cardio-acceleration. Abscissa: total dose in mg. present in the heart-lung preparation. Solid circles, dihydrosolasodanol (5 experiments). Open squares, dihydrosolasodenol (3 experiments). For further explanation, see text.

* The *l*-adrenaline tartrate which was used in these studies was generously supplied by Winthrop Chemical Company.

RESULTS

Dihydrosolasodenol and dihydrosolasodanol

In order to establish more correctly the anti-accelerator potency of dihydrosolasodenol and dihydrosolasodanol additional experiments were performed with the two substances similar to the experiment of Fig. 5 of the earlier communication (Krayer and Briggs, 1950). The results of five experiments with dihydrosolasodanol and of three with dihydrosolasodenol are given in Fig. 1. There appears to be no distinct difference between the potency of the two compounds. The dose-response curve drawn corresponds to the best fitting straight line of a probit plot of all the inhibition values of the eight experiments. It represents an I_{50} value of 1.8 mg.

The maximal amount of 5 per cent dextrose solution given in these eight experiments, with the dose causing complete inhibition of acceleration, varied between 10 and 40 c.c. (average 24 c.c.).

Solasodine and solasodanol

In the attempt to determine quantitatively the anti-accelerator potency of solasodanol difficulties were met on account of the low solubility and low potency of the substance; this required large amounts of 5 per cent dextrose to be administered to the HLP.

In order to assess the influence upon the accelerated heart rate of the solvent itself, five control experiments were run, starting out with a total blood volume in the HLP of 650 to 700 c.c. Adrenaline infusion was carried out as in all other experiments. When the steady state condition of cardio-acceleration was reached, 5 per cent dextrose solution was successively administered in doses of 10, 20, 70, 100, and 100 c.c. up to a total dose of 300 c.c. The injections were made in 1, 2, 4, 5, and 5 min. respectively. Ten minutes were allowed to elapse between the end of one injection and the beginning of the next. This time interval usually sufficed to re-establish steady state conditions of heart rate. Care was taken to maintain the temperature of the blood in the HLP constant.

The injection of 10 c.c. of dextrose solution did not cause a change in heart rate, but when the next dose of 20 c.c. was injected the heart rate decreased between 1.4 and 14.5 (average 7.5) per cent of the acceleration caused by the adrenaline infusion. The additional doses decreased the heart rate further. After the total dose of 300 c.c. had been administered the heart rate in the five experiments stabilized at a level 17, 18, 20, 34, and 38 (average 25.4) per cent below the level of maximal acceleration, in spite of the continuing administration of adrenaline at the same rate.

As the solubility of the hydrochlorides of solasodine and solasodanol in 5 per cent dextrose solution was 0.25 mg. per c.c., not more than between 50 and 75 mg. of either substance was administered in a single experiment.

The results of two experiments with solasodine and of three experiments with solasodanol are shown in Fig. 2, on the left. The per cent values of inhibition are plotted against the doses of the solanum alkaloids as well as against the doses of 5 per cent dextrose solution given with the respective doses of the alkaloids.

The results of the five control experiments with dextrose solution alone are represented by the hatched area. The average decrease in heart rate caused by

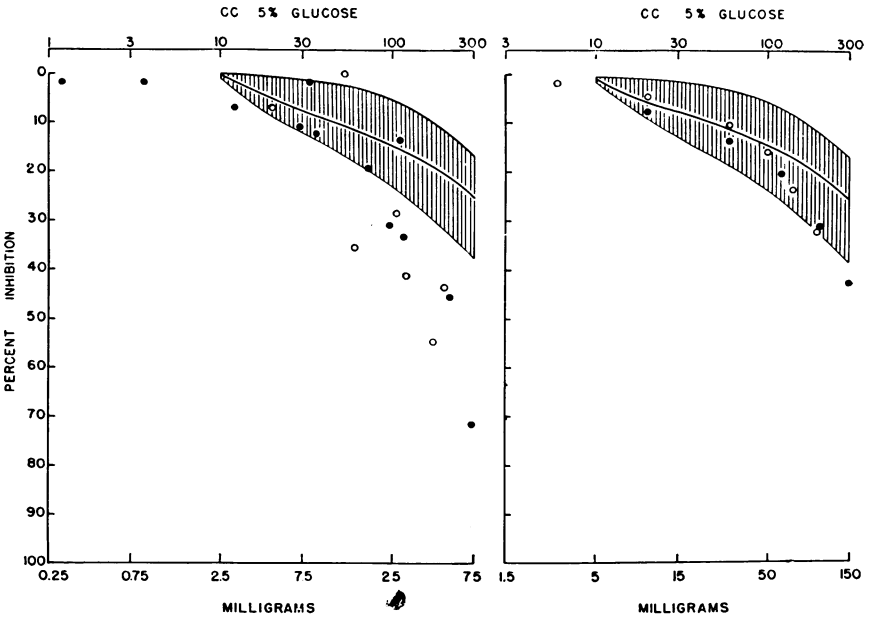

FIG. 2.—The anti-accelerator activity of solasodine, solasodanol, and solanidine. Heart-lung preparation of the dog; 7 experiments. Cardio-acceleration was produced by the continuous infusion of 3 μg. *l*-adrenaline per minute. Ordinate: percentage inhibition of acceleration. Abscissa at the bottom: total dose in mg. present in the HLP. Abscissa at the top: total amount of 5 per cent dextrose solution given with the corresponding total dose of alkaloid. On the left: open circles, solasodine (2 experiments); full circles, solasodanol (3 experiments). On the right: open circles, solanidine "Briggs"; full circles, solanidine "Prelog." One experiment each. Hatched area: heart rate decrease by solvent (5 per cent dextrose) alone. 5 experiments; drawn curve: mean of heart rate decrease. For further explanation, see text.

the solvent in the control experiments is represented by the curve drawn within the hatched area.

It is obvious from these results that doses of approximately 25 mg. of solasodine or solasodanol exert an effect which probably cannot be explained by the action of the solvent. Even doses of 50 to 75 mg. do not cause complete inhibition of acceleration. There appears to be no distinct difference between the potency of solasodine and of solasodanol. While the anti-accelerator activity of the two substances has thus been established by these experiments, the influence of the solvent alone upon the heart rate does not justify attributing validity of an accurate quantitative nature to the results.

Solasonine and solmargine

The glycosides solasonine and solmargine possess the characteristic anti-accelerator property. The onset of action and the rate of development of the maximal effect are fast (see Fig. 3) as with their genin solanidine and with the other three secondary solanum alkamines.

It was pointed out (Krayer, 1949) that the determination of the initial rate presented a problem if anti-accelerator activity was to be measured quantitatively.

In a number of experiments of a given series, run under identical conditions, the inhibition of acceleration decreased the rate below the observed initial rate. In such experiments the " corrected initial rate " was used for the calculation of the percentage inhibition of acceleration caused by a certain dose of an active substance.

The low potency of solasonine and solmargine, in spite of adequate solubility, made it impossible for lack of material to effect complete inhibition of acceleration.

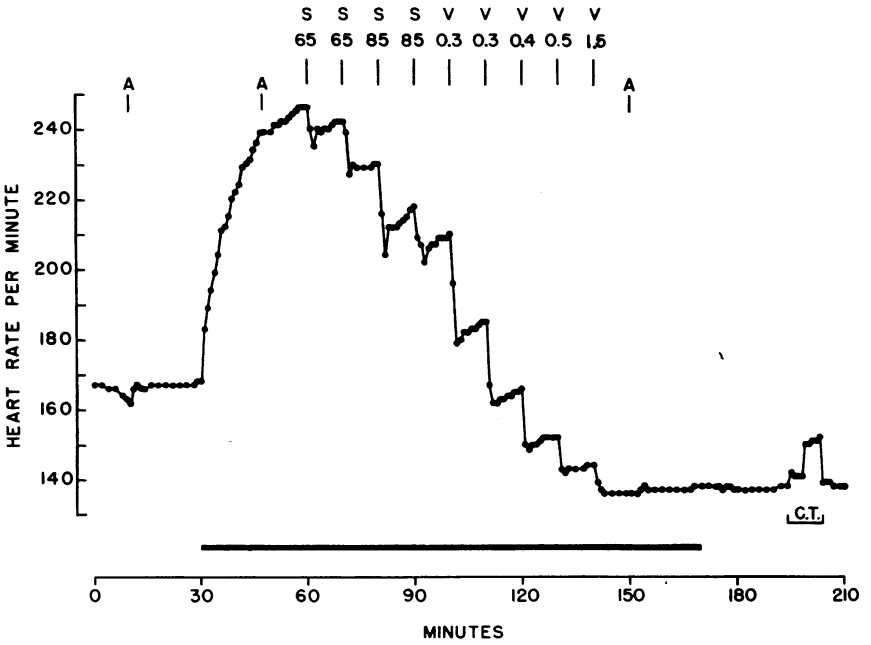

FIG. 3.—The graded anti-accelerator action of solmargine (and veratramine). Dog, male, 12.2 kg. Heart-lung preparation. Mean arterial pressure 103 mm. Hg. Systemic output 550 c.c. per minute. Total blood volume at beginning 850 c.c. Temperature 38.0° C. Black bar: continuous infusion of 3 micrograms of *l*-adrenaline base per minute. A: 10 mg. atropine sulphate were injected. S: solmargine was injected at the signals in doses in mg. indicated by the figures. V: veratramine was injected at the signals in doses in mg. indicated by the figures. At CT a competence test was performed.

The anti-accelerator action was therefore increased by supplementary doses of veratramine.* Veratramine was chosen not only for its potency but also because it is the best known anti-accelerator substance and is available in sufficient quantity to serve as a reference standard for a comparative study of anti-accelerator potency.

The successive administration of solmargine in the experiment of Fig. 3, leading to a total dose of 300 mg., caused a decrease in heart rate from 244 to 210. The subsequent administration of veratramine (up to a total of 3 mg.) reduced the rate to 137 per min. On the basis of the " corrected initial rate " of 137, the inhibition of acceleration caused by 300 mg. of solmargine, therefore, was not 36 beats of an

* The veratramine used was prepared by Dr. S. Bernstein and was generously supplied by Lederle Laboratories, a Division of American Cyanamid Company.

acceleration of 78 (168 to 246) but of a "corrected" acceleration of 109 (137 to 246) or 33 per cent rather than 46 per cent.

The corrected inhibition values of the experiment of Fig. 3 with solmargine and of one experiment conducted with solasonine are given in Table II in terms of

TABLE II

ANTI-ACCELERATOR CARDIAC ACTION OF THE SOLASODINE GLYCOSIDES SOLMARGINE AND SOLASONINE IN THE HEART-LUNG PREPARATION OF THE DOG*

Total dose in mg.		Inhibition per cent	Total dose in mg.		Inhibition per cent
Solmargine	Solasodine		Solasonine	Solasodine	
65	31	4	64.5	30	16
130	62	15	129	60	25
215	102	26	215	100	32
300	143	33	310	140	37
			405	186	44

* Cardio-acceleration was produced by continuous infusion of 3 μg. *l*-adrenaline base per minute.

the genin (solasodine) equivalents of the two glycosides. The total amount of 5 per cent dextrose administered was 3 c.c. in the solmargine and 40 c.c. in the solasonine experiment.

Solanidine

The results of the two experiments with the tertiary amine solanidine are shown in Fig. 2, on the right. All the inhibition values, except one obtained with the total dose of 1_{50} mg. which lies slightly below, fall within the hatched area. The decrease in heart rate, therefore, can be explained by the effect of the solvent alone. Hence, even in a dose of 150 mg. solanidine did not exert a distinct anti-accelerator action under the conditions of these experiments.

Discussion

In spite of the difficulties presented by low solubility and low potency, the results of this study indicate that solasodine has anti-accelerator properties. While no correct I_{50} value could be established, this value can be said to be higher than 50 mg. on the basis of the experiments with solasodine itself. The potency of the solasodine glycosides, solasonine and solmargine, suggest an I_{50} value of the aglycone of more than 180 mg., unless the anti-accelerator property is changed, as is the solubility, by the introduction of the three sugars into the molecule.

The hydrogenation of solasodine leading to solasodanol has no apparent influence upon the anti-accelerator potency, while the opening of the oxide linkage at C_6 leading to dihydrosolasodenol greatly increases it. Further hydrogenation in Ring B, as it takes place in the formation of dihydrosolasodanol from dihydrosolasodenol, is apparently without additional influence on anti-accelerator potency.

The quantitative comparison of the solanum alkaloids with the veratrum alkaloids, veratramine and jervine, examined under comparable conditions in the HLP, yields the following results: solasodine and solasodanol (I_{50} probably considerably above 120 μmoles and possibly higher than 400 μmoles) are far less potent

than jervine ($I_{50} = 18$ μmoles); dihydrosolasodenol and dihydrosolasodanol ($I_{50} = 4.3$ μmoles) are intermediate in potency between jervine and veratramine ($I_{50} = 0.4$ μmoles).

Veratrosine and pseudojervine, the glycosides of veratramine and jervine with one molecule of D-glucose, were shown to have an anti-accelerator action differing from that of their genins in that it develops gradually (Krayer, 1950). In contrast to this, the anti-accelerator action of solasonine and solmargine, glycosides of solasodine and three molecules of sugar, bring about their effect abruptly as does their genin.

The observation that solanidine in doses up to 150 mg. does not exhibit distinct anti-accelerator action is in agreement with further experiments with the tertiary veratrum alkamine cevine, which did not show anti-accelerator action in doses up to 200 mg. The anti-accelerator activity of the solanum as well as of the veratrum alkaloids appears to be restricted to the secondary amines.

SUMMARY

Solasodine, a steroid alkaloid of secondary amine character; its hydrogenation products, solasodanol, dihydrosolasodenol, and dihydrosolasodanol; two of its naturally occurring glycosides, solasonine and solmargine; and the tertiary amine solanidine have been studied for their anti-accelerator potency in the heart-lung preparation of the dog. Acceleration of heart rate was produced by continuous infusion of 3 μg. of *l*-adrenaline base per min. under comparable conditions of blood volume, temperature, and work load.

The secondary solanum alkaloids, including the solasodine glycosides, have anti-accelerator properties similar to those of veratramine and jervine, the secondary veratrum alkamines to which the solanum alkaloids are chemically related.

The potency of solasodine is very low. No change in potency occurs by the hydrogenation of solasodine to solasodanol, while potency is greatly increased by hydrogenation to dihydrosolasodenol and dihydrosolasodanol.

The tertiary amine solanidine appears to have no anti-accelerator activity.

We wish to thank Mr. Arthur C. Campbell for the expert technical help given during the course of these experiments.

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