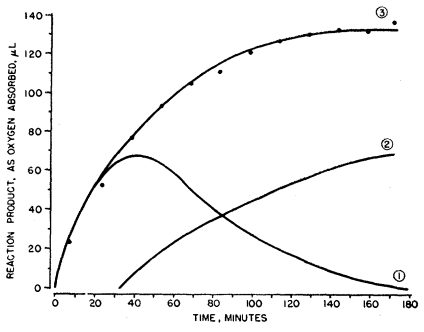

Fig. 1. Oxidation of serotonin in the presence of ceruloplasmin. Reaction rates were derived from spectrophotometric and oxygen-consumption data. Curve 1, product of the first oxidation (p_1); curve 2, product of the second oxidation (p_2); curve 3, total oxygen consumption ($p_1 + 2p_2$); the dots show observed oxygen consumption.

Ceruloplasmin concentrates were obtained by further alcohol and salt fractionation of Cohn's fraction IV-1, method 6 (*4*), from pooled human plasma. Preparation 49, which was used in the experiments described here, contained 0.23 g of copper and 1.23 g of protein per 100 ml. Thus, the enzyme had a purity above 50 percent (*2*). However, its catalytic properties were identical with those of more highly purified (90 percent) concentrates.

Three-tenths of a milliliter of enzyme solution and 2.2 ml of 0.1*M* acetate buffer, *p*H 6.00 ± 0.01, were placed in Warburg flasks and equilibrated at 37°C for 15 minutes before 0.5 ml of serotonin creatinine sulfate (6 μmole) was tipped in. The reaction was allowed to proceed in air for various intervals and was then stopped by the addition of 1 ml of 10 percent trichloroacetic acid. Spectra of suitably diluted filtrates were determined on the Beckman DU spectrophotometer.

The characteristic ultraviolet absorption of serotonin rapidly diminished, becoming eventually about half as intense as it was initially. In the visible region, a broad absorption band centered at about 530 mμ developed, but this was later obscured by more rapidly increasing absorption at shorter wave lengths. In this, as in other experiments with varying concentrations of enzyme and substrate, two atoms of oxygen per molecule of substrate were consumed. The shape of oxygen consumption and time-absorbance curves suggested that two consecutive oxidation steps might be occurring and that the second step gained no appreciable velocity until the first was essentially complete. Therefore, for mathematical analysis of the data, it was assumed that, during the earlier part of the oxidation, the only species present which absorbed visible light was the first oxidation product, P_1. Relating oxygen consumption to absorbance yielded extinction coefficients at two wavelengths for P_1. Here,

$$A_{400} = \varepsilon_{1\text{-}400}\, p_1$$

and

$$A_{530} = \varepsilon_{1\text{-}530}\, p_1$$

where A is absorbance, $\varepsilon_{1\text{-}400}$ and $\varepsilon_{1\text{-}530}$ are extinction coefficients at $\lambda = 400$ mμ and 530 mμ, respectively, and p_1 is observed oxygen consumption in microliters.

At completion, when only the second product P_2 was presumably present, oxygen consumption was related to absorbance to obtain extinction coefficients for P_2. In this case

$$A_{400} = \varepsilon_{2\text{-}400}\, p_2'$$

and

$$A_{530} = \varepsilon_{2\text{-}530}\, p_2'$$

where p_2' is one-half of the observed oxygen consumption at completion of the reaction and is equal to one atom of oxygen per molecule of serotonin originally present.

At any time during the oxidation

$$A_{400} = \varepsilon_{1\text{-}400}\, p_1 + \varepsilon_{2\text{-}400}\, p_2$$

and

$$A_{530} = \varepsilon_{1\text{-}530}\, p_1 + \varepsilon_{2\text{-}530}\, p_2$$

Simultaneous solution of these equations gave values for p_1 and p_2 in terms of microliters of oxygen absorbed; these values are plotted against time in Fig. 1. Total oxygen consumed up to any time would equal the amount of P_1 present (p_1) plus twice the amount of P_2 present ($2p_2$) at that time. This sum yielded the calculated oxygen consumption curve in Fig. 1, which fits the observed values well.

By varying the ratio of enzyme to substrate, it was possible to estimate the velocity constant and Michaelis' constant for the first reaction. From the rate of appearance of P_2 shown in Fig. 1, these constants for the second reaction were calculated. In this way it was found that the first reaction was about twice as rapid as the second and that the affinity of the enzyme for serotonin was some 3.6 times as great as it was for the first oxidation product. Since Michaelis' constant is not identical with the association constant, this latter figure is at best a rough estimate of the true ratio of affinities.

Ceruloplasmin has no monoamine oxidase activity. Neither does it catalyze the oxidation of monoamine oxidase substrates such as tyramine and phenethylamine, nor does it liberate ammonia in the course of the catalyzed oxidation of serotonin.

Like serotonin, 2-methylserotonin absorbed 2 atoms of oxygen per molecule, which indicates that the 2-position is probably not a point of oxidative attack. Polymerization of oxidized serotonin to melaninlike pigments is not likely in view of the report (*5*) that only benzene-oxygenated indoles which are unsubstituted in position 3 react in this way.

The oxidation of serotonin exhibits certain similarities to the tyrosinase-catalyzed oxidation of catechol (*6*), where oxidation to *o*-quinone is followed by hydroxylation. The two reactions differ in several respects, one being that in the tyrosinase-catechol reaction, more than two oxygen atoms can be consumed, depending upon the ratio of enzyme to substrate (*7*).

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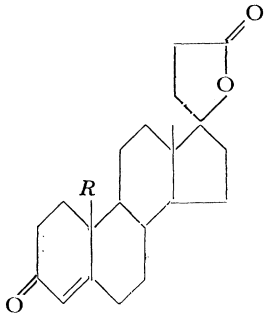
26 June 1957

Action of New Steroids in Blocking Effects of Aldosterone and Deoxycorticosterone on Salt

Aldosterone may be of etiological significance in salt and water retention of congestive heart failure, nephrosis, liver cirrhosis, and toxemia of pregnancy (*1*). We wish to report aldosterone-blocking activity of 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl)propionic acid γ-lactone (SC-5233) and its 19-nor analog (SC-8109 (*2*). Such compounds may serve as useful agents in elucidating the role of aldosterone in edema and may be of value in the treatment of edema. Structures of the steroids are as follows:



In steroid SC-5233, R is CH_3; in steroid SC-8109, R is H. Results demonstrating aldosterone-blocking activity with SC-5233 are summarized in Fig. 1. Aldosterone in a solution of ethanol in 0.86-percent sodium chloride (20/80 by volume) was subcutaneously injected into adrenalectomized rats, either alone or with oil solutions of SC-5233. Four-hour samples of urine were collected from individual animals for sodium and potassium analyses. Aldosterone alone caused a reduction in the value of the Na/K ratio in the urine, but in the presence of 1.2 and 4.8 mg of SC-5233 per rat this reduction was significantly reversed.

The 19-nor analog, SC-8109, similarly blocked aldosterone activity. For example, administration of 0.98 μg of aldosterone reduced the Na/K ratio to 0.63 ± 0.07 (mean ± standard error). A dose of 1.3 mg of SC-8109 significantly counteracted this effect, giving a ratio of 1.12 ± 0.12. We attach strong importance to these findings because SC-5233 and SC-8109 appear to represent the first known examples of aldosterone blockers.

Several studies were undertaken to define the mechanism of blocking with deoxycorticosterone acetate (DOCA). We used this compound because of its availability, structure, electrolytic effects, and possible similarity in mechanism of action to aldosterone. The results obtained with DOCA and SC-5233 are summarized in the succeeding paragraphs; those obtained with SC-8109 are incomplete (*3, 4*).

A progressive blocking of the action of 12 μg of DOCA on the Na/K ratio was obtained with six different doses of SC-5233, ranging from 0.15 to 4.8 mg. These data showed that approximately 0.24 mg of SC-5233 was required for a 50-percent block of DOCA (*5*). Progesterone, which was recently described as a DOCA-blocker in man (*6*), showed similar activity with a dose of 1.8 mg. Compound SC-8109 was effective at 0.067 mg. By comparison of doses, progesterone, SC-5233, and SC-8109 showed relative activities of 1, 7.5, and 26.8, respectively.

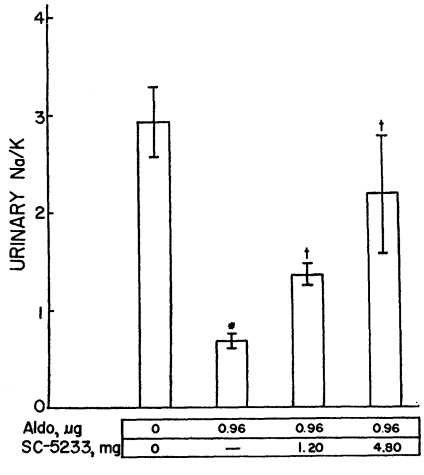


Fig. 1. Effects of SC-5233 in blocking urinary Na/K action of aldosterone in adrenalectomized rats (12 animals per treatment). Standard error is shown by vertical lines. * $P < 0.05$, compared with no treatment; † $P < 0.05$, compared with treatment with aldosterone alone.

Table 1. Effects of various doses of SC-5233 in blocking the action of deoxycorticosterone acetate (DOCA) in adrenalectomized rats.

Treatment (dose per rat)		No. of rats	Urinary Na/K (mean ± S.E.*)
SC-5233 (mg)	DOCA (μg)		
	24	20	0.47 ± 0.04
	48	19	0.54 ± 0.05
0.6	24	9	0.80 ± 0.13
1.2	48	9	0.81 ± 0.04
2.4	24	9	1.35 ± 0.36
4.8	48	9	1.23 ± 0.15
9.6	24	9	1.54 ± 0.40
19.2	48	8	1.62 ± 0.19

* S.E. = standard error.

Table 2. Effects of large amounts of deoxycorticosterone acetate (DOCA) in overcoming blocking action of SC-5233 in adrenalectomized rats.

Treatment (dose per rat)		Urinary Na/K (mean ± S.E.*)
SC-5233 (mg)	DOCA (μg)	
2.4	48	1.09 ± 0.13
2.4	240	0.75 ± 0.13
2.4	1200	0.69 ± 0.16
	48	0.54 ± 0.10

* S.E. = standard error; 15 rats per treatment; 20 untreated controls showed a Na/K response of 1.90.

The effects of SC-5233 in counteracting reduction of the Na/K ratio produced by 24 and 48 μg of DOCA were investigated at two dosage levels and at various ratios (Table 1). Doubling the dose at a fixed ratio of SC-5233/DOCA did not cause significant changes in Na/K values. Increasing ratios of SC-5233/DOCA, however, progressively blocked the effects of 24 and 48 μg of DOCA. These observations suggest that (i) equal blocking results with similar ratios and (ii) blocking increases with larger ratios of SC-5233/DOCA. It would appear that SC-5233 acts as a blocker according to the law of mass action.

The opposite question of reversing the action of SC-5233 with an excess of DOCA was studied (Table 2). The results indicate that the effects of 2.4 mg of SC-5233 were reversed with 240 and 1200 μg of DOCA. We feel that these results demonstrate reversible competition.

In order to rule out the possibility that SC-5233 had a direct effect of increasing the urinary Na/K ratio, instead of specifically blocking DOCA, we performed the following experiment. Doses of 0.0, 1.2, 9.6, and 19.2 mg of SC-5233 alone were given; these doses produced Na/K ratios of 2.15, 2.38, 2.67, and 2.32, respectively (nine rats per treatment). None of the responses with SC-5233 treatment significantly exceeded the control response of 2.15. Increases in the Na/K ratio greater than 0.66 above the control value would occur by chance once in ten trials, whereas in our results the largest increase was 0.52. Thus, SC-5233 does not in itself greatly affect the urinary Na/K ratio.

The results of these studies strongly suggest that SC-5233 and, possibly, SC-8109 exert their effects on electrolytes by competition with DOCA and aldosterone (*7*).

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3. A more detailed report on these investigations and other pharmacological effects of SC-5233 and SC-8109 is in preparation.
4. When given orally, 4.8 mg of SC-8109 significantly reversed the Na/K effect of 12 μg of DOCA from 0.59 ± 0.05 (mean ± standard error) to 0.95 ± 0.14. At the same oral dose, SC-5233 was inactive.
5. The term *percent block* refers to values obtained by the following formula: (net blocking effect of test compound) × 100/(effect of DOCA).
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7. We gratefully acknowledge the valuable technical assistance of Marjorie A. Thomas and Elizabeth D. Griffin. Thanks are due to David W. Calhoun for the statistical work.

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Sodium Diuresis Induced by Steroidal Antagonists of Aldosterone

For several years it has been known that the adrenal cortex secretes a number of steroidal hormones which increase the tendency of the renal tubules to reabsorb sodium. The adrenal steroid of most importance in the physiological regulation of electrolyte metabolism is aldosterone (*1*). Aldosterone has been shown to play a crucial role in normal physiology in promoting the conservation