GLYCYRRHETINIC ACID—A TRITERPENE WITH ANTI-OESTROGENIC AND ANTI-INFLAMMATORY ACTIVITY*

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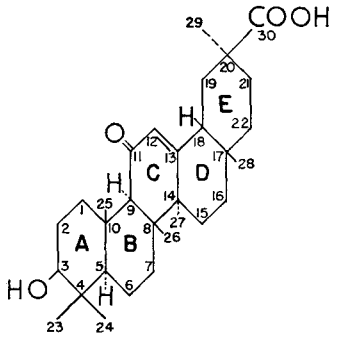
Glycyrrhetinic acid was found to resemble the Δ^4-3-ketosteroids in antagonising uterine growth response to exogenous oestrogen in doses which did not interfere with somatic growth or the oestrogenic effect on pituitary trophic hormones. The anti-oestrogenic effect was not mediated through gonadal inhibition, adrenal stimulation or potentiation of endogenous adrenal corticoids. Glycyrrhetinic acid is neither androgenic, oestrogenic nor anti-androgenic. However, it does resemble the glucocorticoids in depressing granuloma tissue formation in adrenalectomised rats.

GLYCYRRHETINIC acid is the aglycone[1] of glycyrrhizinic acid, the constituent of crude licorice extract affecting electrolyte balance[2–4]. Glycyrrhetinic acid is a triterpene[5] resembling allopregnane and androstane in the orientation of the hydrogen atom on C(5). (I). The $\alpha\beta$-unsatura-



(I)

ted 11 ketone group in ring C morphologically relates glycyrrhetinic acid to the Δ^4-3-ketosteroids (testosterone, progresterone, adrenal corticoids). The similarity in chemical configuration between glycyrrhetinic acid and corticoids is probably the basis for the mineralocorticoid activity[4,6,7] and pituitary-adrenal inhibition[2,8] previously reported. Derivatives of glycyrrhetinic acid which had anti-inflammatory action in the intact animal[7,9] did not influence liver glycogen deposition[10].

This report is concerned with the effect of glycyrrhetinic acid on the inhibition of growth induced by a steroid of the oestrane series, an action of the Δ^4-3-ketosteroids[11–14], and the anti-inflammatory activity of the acid in the absence of the adrenal gland.

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METHODS

CFN rats, maintained on Purina chow, were used. Normal rats received water *ad libitum*, while adrenalectomized animals received 1 per cent saline instead. Drugs were administered subcutaneously either in a vehicle of 1 part ethanol and 9 parts sesame oil for β-glycyrrhetinic acid* and glycyrrhetinic acid,† or in sesame oil for the sex hormones.‡

β-Glycyrrhetinic Acid and Sex Hormones

Rats, 22 to 23 days old, weighing 35 to 40 g., were treated for three days. Each rat received two injections (at different sites) twice daily of 0·1 ml. of vehicle with or without β-glycyrrhetinic acid as well as sesame oil with sex hormone. All doses were expressed as the total dose administered over 3 days. 72 to 75 hours after the first dose the animals were killed by decapitation. Organs were weighed on the Roller-Smith balance, and a comparison of relative weights was made by analysis of variance.

The increase in uterine weight in intact and bilaterally adrenalectomised rats (adrenalectomised 1 hour before the first dose) to 0·30 μg. of oestradiol benzoate was determined with and without β-glycyrrhetinic acid. Intact rats received a total of 0·60 to 6·0 mg., and adrenalectomised rats 6·0 mg. of β-glycyrrhetinic acid.

The effect of β-glycyrrhetinic acid on the response of the uterus to oestradiol benzoate was assessed in a 6-point assay. The oestradiol dose response curve of vehicle-treated rats was compared with that for the animals treated with β-glycyrrhetinic acid (6·0 mg.) for parallelism and potency.

Studies were then made to determine whether the anti-oestrogenic effect was limited to the uterus.

Somatic growth of immature female rats which had been individually housed was assessed by weighing the animals before and after the period of treatment. The adrenals, thymus and ovaries were weighed in addition to the uterus. The four groups each of ten individually housed rats consisted of controls, oestradiol-treated (0·30 μg.), β-glycyrrhetinic acid treated (6·0 mg.), and oestradiol (0·30 μg.) plus β-glycyrrhetinic acid (6·0 mg.) treated animals.

The effect of β-glycyrrhetinic acid on testicular atrophy induced by 75 μg. of oestradiol benzoate in immature male rats was also determined. Weights of the testes of controls, oestradiol treated, and oestradiol and β-glycyrrhetinic acid (6·0 mg.) treated animals were compared.

To determine whether the acid had androgenic or anti-androgenic activity, seminal vesicle and testes weights of rats treated with testosterone

* Supplied by S. B. Penick and Co., N.Y.

† Supplied by Dr. T. E. Weichselbaum, Washington University Medical School, St. Louis, Missouri. (m.p. 275°; $[\alpha]_D$ + 112°; solubility at room temp., 20 mg./ml. in ethanol.) *See* Carlat, Margraf, Weathers and Weichsel baum, *Proc. Soc. exp. Biol., N.Y.*, 1959, **102,** 245.

‡ Estradiol benzoate and testosterone propionate supplied by Organon Inc., Nutley, N.J.

propionate (1·20 mg.), β-glycyrrhetinic acid (6·0 mg.), testosterone propionate (1·20 mg.) and β-glycyrrhetinic acid (6·0 mg.) were compared with controls.

Depression of Granuloma Tissue Formation in Bilaterally Adrenalectomised Rats

The anti-inflammatory action of β-glycyrrhetinic acid and glycyrrhetinic acid (in total doses of 4·0 and 8·0 mg.) was measured by depression of granuloma tissue formation by the cotton pellet technique[15]. Two cotton pellets (10 to 12 mg.) were subcutaneously implanted in the dorsal region

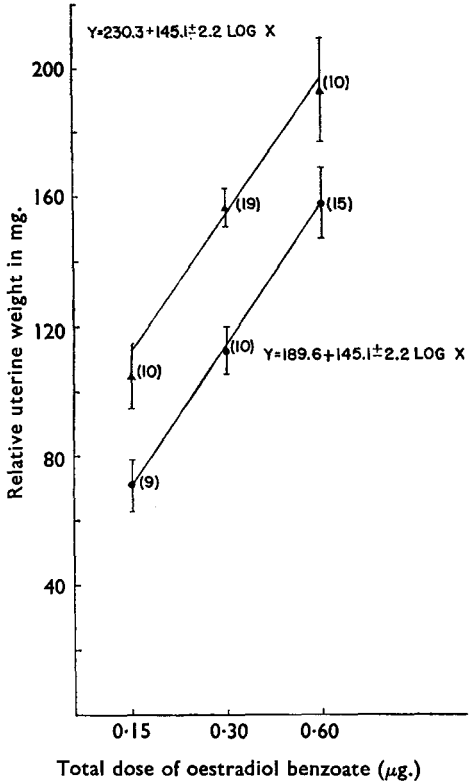

FIG. 1. Dose response curve for oestradiol benzoate in immature rats based on relative uterine weights. Oestradiol and β-glycyrrhetinic acid or vehicle administered subcutaneously for 3 days in 6 doses. Potency of oestradiol in the presence of 6·0 mg. of β-glycyrrhetinic acid (total dose) is 0·53 ± 0·05 ($P < 0.001$ based on analysis of variance).

▲ Oestradiol. ● Oestradiol + 6 mg. of β-glycyrrhetinic acid.

of male rats of 100 g. weight at the time of bilateral adrenalectomy. Either the acid in 1 ml. of vehicle or the vehicle alone was administered for 4 days after adrenalectomy. Pellets with granulation tissue were

removed 24 hours after the last dose, oven dried overnight at 80°, and weighed.

RESULTS

β-Glycyrrhetinic Acid and Sex Hormones

The increase in weight of the uterus in response to exogenous oestrogen indicated that β-glycyrrhetinic acid was a potent oestrogen antagonist (Fig. 1, Table I). β-Glycyrrhetinic acid in doses from 1·20 to 6·0 mg. produced a 23 to 33 per cent decrease in the weight response of the uterus to 0·30 μg. of oestradiol benzoate with no significant differences between

TABLE I

DEPRESSION BY GLYCYRRHETINIC ACID OF THE UTERINE WEIGHT RESPONSE OF IMMATURE RATS TO 0·3 μg. OF OESTRADIOL BENZOATE

	Total dose in mg.	No. of animals	Uterine weight mg./100 g.	Per cent decrease	P value
Intact	Control	19	156·9 ± 6·2	—	—
	0·6	10	141·0 ± 9·0	10·1	N.S.
	1·2	6	105·0 ± 10·1	33·1	<0·01
	1·8	6	120·0 ± 12·2	23·5	<0·02>0·01
	2·4	4	104·6 ± 9·9	33·3	<0·01
	3·0	5	107·7 ± 4·5	31·4	<0·001
	6·0	10	113·0 ± 7·3	30·0	<0·001
Adrenalectomised ..	Control	9	143·1 ± 11·1	—	—
	6·0	9	109·4 ± 4·4	23·5	<0·001

doses (Table I). This decrease in response quantitatively agrees with the previously reported results for progesterone and desoxycorticosterone[12]. Bilateral adrenalectomy 1 hour before the first dose did not affect the anti-oestrogenic potency of 6·0 mg. of β-glycyrrhetinic acid (Table I). There were no significant differences between the responses of intact and adrenalectomised rats similarly treated (Table I).

TABLE II

THE EFFECT OF GLYCYRRHETINIC ACID ON THE UTERINE WEIGHTS OF IMMATURE RATS

Total dose in mg.	No. of animals	Uterine weight mg./100 g.
Control	17	41·2 ± 4·5
1·2	5	55·9 ± 2·5
1·8	5	54·6 ± 3·5
2·4	5	57·4 ± 7·4
3·0	5	38·3 ± 2·1
6·0	11	36·9 ± 10·0
15·0	5	52·5 ± 5·1
30·0	4	37·3 ± 8·6

A comparison between the dose response curves of rats given oestradiol alone and those given also β-glycyrrhetinic acid (Fig. 1) indicated that 6·0 mg. of the acid caused a significant reduction in the potency of the oestradiol, twice the dose of which was required in the presence of the acid to obtain a response equivalent to that of the oestrogen alone.

β-Glycyrrhetinic acid had no effect upon the immature uterus in the absence of exogenous oestrogen (Table II). There were no significant

differences between the uterine weights of untreated and glycyrrhetinic acid treated rats.

β-Glycyrrhetinic acid restricted the uterine weight increase in response to oestrogen in doses which did not interfere with somatic growth or depress gonadal size (Table III). Moreover, adrenal hypertrophy and thymic involution induced by 0·30 μg. of oestradiol benzoate were not affected by the acid (Table III). Testicular atrophy (Table IV) induced

TABLE III

A COMPARISON OF INCREASES IN BODY WEIGHT AND RELATIVE ORGAN WEIGHTS OF IMMATURE FEMALE RATS (10 PER GROUP) TREATED WITH 0·3 μg. OF OESTRADIOL BENZOATE OR 6 mg. OF GLYCYRRHETINIC ACID OR BOTH

Group No.	Oestradiol benzoate μg.	Glycyrrhetinic acid in mg.	Increase in weight	Adrenals mg./100 g.	Thymus mg./100 g.	Ovaries mg./100g.
1	Control	Control	8·5	20·6 ± 1·00	235·1 ± 13·68	18·1 ± 0·65
2	Control	6	10·2	20·1 ± 1·00	281·9 ± 25·70	17·9 ± 1·11
3	0·3	Control	8·0	23·4 ± 1·43	185·5 ± 20·20	16·5 ± 0·72
4	0·3	6	7·4	27·1 ± 2·00	149·1 ± 4·36	16·0 ± 0·65

TABLE IV

THE EFFECT OF GLYCYRRHETINIC ACID ON THE SIZE OF THE TESTES OF IMMATURE RATS GIVEN VEHICLE , TESTOSTERONE PROPIONATE OR OESTRADIOL BENZOATE

Group No.	Hormone and dose	Glycyrrhetinic acid in mg.	No. of animals	Testes mg./100 g.
1	Sesame oil only	Vehicle only	12	592·8 ± 18·0
2	" " "	6	4	669·6 ± 64·6
3	Testosterone 1·20 mg.	Vehicle only	16	594·4 ± 24·6
4	Testosterone 1·20 mg.	6	8	568·1 ± 22·9
5	Oestradiol 75 μg.	Vehicle only	7	319·6 ± 17·5*
6	Oestradiol 75 μg.	6	8	439·1 ± 15·5

* Decrease in testes size significant, P = 0·02.
† No significant difference from group 5.

TABLE V

EFFECT OF GLYCYRRHETINIC ACID ON THE RESPONSE OF THE SEMINAL VESICLES TO TESTOSTERONE IN IMMATURE RATS

Group No.	Testosterone propionate mg.	Glycyrrhetinic acid in mg.	No. of animals	Relative weight of seminal vesicles mg.
1	Sesame oil only	Vehicle only	4	20·8 ± 2·9
2	" " "	6	4	24·7 ± 2·7
3	1·2	Vehicle only	8	39·3 ± 2·4
4	1·2	6	8	35·9 ± 1·6

by 75 μg. of oestradiol benzoate was also not affected by 6·0 mg. of β-glycyrrhetinic acid. Apparently the drug did not antagonise the effect of oestrogen upon the pituitary-trophic hormones.

β-Glycyrrhetinic acid had no androgen activity (Table V) in immature rats nor did it interfere with the proliferative response of the seminal vesicle to 1·20 mg. of testosterone propionate. It also had no effect upon the size of the immature testes (Table IV).

Depression of Granuloma Tissue Formation in Bilaterally Adrenalectomised Rats

The two preparations (β-glycyrrhetinic acid and glycyrrhetinic acid) significantly depressed granulation tissue formation in doses of 4·0 and 8·0 mg. (Table VI). The differences in decreased granulation tissue formation between doses as well as between preparations were not significant.

TABLE VI

DEPRESSION OF GRANULOMA FORMATION BY GLYCYRRHETINIC ACID IN THE COTTON PELLET TEST IN ADRENALECTOMISED MALE RATS

Treatment	Total dose mg.	No. of pellets	Mean granuloma dry weight mg	Per cent decrease	P value
Controls	—	23	21·1 ± 2·00	—	—
β-Glycyrrhetinic acid ..	4	12	9·9 ± 0·86	53·1	<0·001
" " ..	8	10	8·6 ± 0·91	59·2	<0·001
Glycyrrhetinic acid	4	11	13·6 ± 1·48	35·3	<0·01
" "	8	22	13·5 ± 2·45	35·8	<0·02>0·01

DISCUSSION

Glycyrrhetinic acid resembles the Δ^4-3-ketosteroids in antagonising oestrogen. β-Glycyrrhetinic acid was found to be a potent inhibitor of oestrogen-induced uterine growth in doses which did not interfere with somatic growth nor the oestrogenic effect on pituitary trophic hormones.

The antagonism of oestrogen-induced uterine growth does not appear to be mediated through gonadal inhibition, adrenal stimulation or potentiation of adrenal corticoid action. This effect of the acid was not altered by adrenalectomy. It is unlikely therefore that the observed action in depressing oestrogen-induced growth of the uterus was due to the ability of the acid to interfere with the metabolism of hydrocortisone[16] and desoxycorticosterone[17]. The antagonism may be through the same mechanism suggested for the naturally occurring steroids. Testosterone, progesterone and adrenal corticoids antagonise the oestrogenic response of the uterus by alteration of enzyme systems[18–21]; consequently, uterine cell permeability changes induced by oestrogen are affected, and therefore the degree of proliferative response[21].

The two parallel dose response curves obtained with oestradiol benzoate with or without glycyrrhetinic acid indicated that even a 4-fold increase in exogenous oestrogen could not decrease the degree of inhibition induced by 6·0 mg. of β-glycyrrhetinic acid. The anti-oestrogenic effect obtained with the minimum effective dose of the acid (1·20 mg.) was not increased by a 5-fold increase in dosage (to 6·0 mg.). Apparently the restriction of uterine weight response had no relation to the oestradiol-glycyrrhetinic acid ratio.

There has been much speculation about the requisite structural factors in steroid activity. Mardones[22] has reported that oxidation of C(11), C(17) or C(21) diminished anti-oestrogenic potency and increased corticoid potency. When the 3-ketone group was present, neither the

absence of a double bond from testosterone[23] nor the addition of another double bond to cortisone and hydrocortisone[10] altered the anti-oestrogenic activity. However, androstenediol which does not have a ketonic oxygen did not have anti-oestrogenic activity[23]. The ketonic oxygen itself regardless of position may be necessary for anti-oestrogenic activity since its presence in glycyrrhetinic acid endows the acid with the ability to antagonise oestrogen.

Glycyrrhetinic acid resembles the glucocorticoids in anti-inflammatory action, which is not dependent upon the adrenal gland. Depression of granulation tissue in adrenalectomised animals must be attributed to the similarity in chemical configuration between glycyrrhetinic acid and the corticoids. The absence of a correlation between dose and response in this work was probably due to the poor absorption or rapid excretion of the drug or both.

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