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Anabolic and androgenic activities, in rat, of some nandrolone and androstanolone esters‡

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The anabolic and androgenic activities of the formate to undecanoate esters of nandrolone and formate to valerate esters of androstanolone, after intramuscular injection, have been determined in rat. The response to a given dose was measured as cumulative weight (the area under the plot of weight of indicator organ against time). Levator anus muscle was used to assess anabolic activity, and the sum of the cumulative weights for prostate and seminal vesicles for androgenic activity. Log dose-log cumulative weight plots were parallel, and biological activities were expressed as the cumulative weight corresponding to a 2 μM dose, calculated from the regression lines. Anabolic-androgenic ratios for both series were calculated and were found to be minimum in the region of the propionate and butyrate. The anabolic-androgenic ratios of the nandrolone esters continued to increase after the minimum, as the series ascended. This method is believed to give a reliable assessment of anabolic and androngenic activities of steroid esters.

The weight of levator anus muscle in rat is a widely accepted measure of anabolic activity. The original experimental procedure was described by Eisenberg & Gordan (1950), although a modification introduced by Hershberger, Shipley & Meyer (1953) is probably the most commonly used technique. In both methods, castrated rats are injected with the drug under test and levator ani muscles removed at the end of a predetermined period. Anabolic activity is assessed as the dose required to produce a given weight increase, relative to a standard, usually testosterone. All known anabolic agents possess some androgenic activity. Relative anabolic and androgenic effects can be assessed by comparing the responses of levator anus and an androgenic target organ, normally prostate and/or seminal vesicles, and expressed as an anabolic-androgenic ratio.

The relative merits of the various anabolic-androgenic ratios have been discussed by Kruskemper (1968), who suggested the best computation was that given by Overbeek & de Visser (1961).

$$\text{Ratio} = \frac{\text{Anabolic response (sample/standard)}}{\text{Androgenic response (sample/standard)}}$$

It is well known that this ratio can vary with the time after which the organs are removed, particularly when comparing steroid esters having different durations of action. An alternative method of assessing anabolic and androgenic activities has been proposed by Chaudry & James (1974), and is used here to measure anabolic-androgenic ratios in two homologous series of esters.

MATERIALS AND METHODS

Materials

Androstanolone, nandrolone (17-β-hydroxy-19-nor-androst-4-en-3-one) and nandrolone decanoate were gifts from Organon Laboratories Ltd. Formate esters were prepared according to Ringold, Loken & others (1956) (for preparation of 3-β-hydroxyandrost-5-en-17-one formate). The remaining androstanolone esters were obtained by refluxing with the anhydride in the presence of pyridine, and the remaining nandrolone esters were prepared through the acid chloride. Melting points, where published, agreed with the literature; the physical properties of the new compounds are given elsewhere (Chaudry & James, 1974).

Biological activities

Male albino rats (40 to 60 g), were castrated under ether anaesthesia and injected 14 days later, with 0·1 ml of either ethyl oleate (controls), or ester solution in ethyl oleate, into the left gluteus muscle. Four dose levels were tested for each ester. At various time intervals, groups of animals were killed and prostate glands, seminal vesicles and levator ani removed and weighed. Tests were continued until the

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treated organ weights returned to control levels, this required between 18 to 56 days. Biological activities were assessed as cumulative weights, obtained by determining the areas between the plots of organ weights of the control and treated animals, against time. Each cumulative weight was obtained from at least 32 animals.

RESULTS AND DISCUSSION

The effect of various doses of the nandrolone and androstanolone esters upon levator anus and seminal vesicles plus prostate gland is presented in Table 1 as the cumulative weight in mg days. Over the range of doses studied (about 0·6 to 3·3 μM) there was an increasing effect at both anabolic and androgenic sites with increasing dose of each ester. Below this dose range, responses were too small to be recorded accurately and, above it, further increases in dose had little effect. The plots of log dose versus log cumulative weight for both series of esters and for both anabolic and androgenic activities were rectilinear, all giving correlation coefficients exceeding 0·9 (Table 2) as determined by least square analysis.

A limitation of assessing either anabolic or androgenic activity from the weight of an indicator organ at a given time after administration of the hormone, is the requirement that the time-response profiles of sample and standard must be the same. This assumption is rarely justified, so that the assessment usually becomes dependent on the time at which the indicator organ is removed and weighed. A typical example derived from the data in Table 1, is given in Fig. 1 which shows the weights of seminal vesicles after intramuscular injection of 1 mg of either androstanolone acetate or valerate in 0·1 ml of ethyl oleate. The maxima of the time-response plots appear at different times and after 5 days the initial relative androgenic effects of the two esters are reversed. Chaudry & James (1974) have used the area under the time-response curve as a measure of overall anabolic activity. Provided that the experiment is continued until organ weights reach control values, this area (cumulative weight) would be independent of time and is more truly representative of the biological response.

For valid comparisons between compounds it is necessary for the dose-response plots to be parallel, but there appears to be no generally accepted criterion for deciding when they are 'significantly parallel'. Most investigators appear to decide subjectively, by eye, and such an assessment of the results quoted here suggests that the two homologous series give four sets of parallel lines, except the nandrolone decanoate plots. A test for parallelism described by Bailey (1972), was applied to the anabolic responses to nandrolone esters, given in Table 1, and indicated that with the exception of the undecanoate ($P'>0·2$), there is no evidence at the 5% level of significance, that the differences in slope are due to more than chance variation in the results. In an alternative statistical test, the probability that the results represent a family of parallel lines can be tested by assessing the probability that the combined normalised results represent one line, rather than a series of intersecting lines. This test indicated that if the lines are parallel, the chance of a difference between slopes, as great as those observed, is at least 5 %. Exclusion of the undecanoate results increased the

Table 1. *Anabolic and androgenic responses to androstanolone and nandrolone esters.*

	Cummulative wt (mg days)							
	Levator anus				Seminal vesicles + prostate			
Dose (mg)	0·40	0·60	0·80	1·00	0·40	0·60	0·80	1·00
Androstanolone formate	617 (166)		967 (75)	935 (70)	288 (75)	346 (58)	505 (75)	568 (70)
acetate	1068 (144)	1535 (158)	1707 (163)	1594 (121)	516 (55)	769 (82)	887 (97)	975 (64)
butyrate	1174 (166)	1410 (97)	1639 (87)	2071 (109)	622 (52)	786 (76)	885 (78)	952 (70)
valerate	1278 (164)	1719 (177)	2164 (121)	2775 (136)	640 (51)	762 (65)	1022 (51)	1225 (35)
Nandrolone formate	589 (126)	690 (91)	972 (114)	1176 (95)	91 (24)	235 (26)	230 (34)	248 (32)
acetate	647 (87)	777 (106)	1323 (114)	1594 (162)	154 (28)	241 (20)	339 (44)	394 (35)
propionate	884 (112)	1365 (135)	1693 (135)	1880 (96)	302 (31)	391 (39)	466 (40)	501 (24)
valerate	1272 (115)	1744 (96)	2376 (89)	2526 (147)	449 (34)	541 (45)	829 (51)	829 (59)
Dose (mg)	0·25	0·50	0·75	1·00	0·25	0·50	0·75	1·00
Androstanolone propionate	834 (251)	1172 (392)	1445 (219)	1992 (303)	411 (44)	811 (116)	1003 (101)	1921 (167)
Nandrolone butyrate	547 (162)	669 (224)	876 (133)	1488 (234)	115 (27)	247 (46)	433 (48)	640 (40)
hexanoate	968 (413)	2142 (519)	2557 (519)	3731 (651)	252 (58)	632 (78)	885 (86)	1309 (114)
heptanoate	1079 (267)	3193 (583)	3463 (519)	6559 (449)	303 (63)	708 (101)	862 (107)	1385 (140)
octanoate	1063 (363)	2701 (348)	3856 (399)	5557 (395)	256 (30)	477 (29)	758 (36)	1085 (51)
nonanoate	1047 (576)	2509 (573)	2960 (684)	5080 (742)	168 (34)	388 (65)	441 (79)	817 (74)
decanoate	1410 (541)	3192 (502)	4055 (509)	7735 (668)	130 (63)	267 (65)	565 (61)	865 (104)
undecanoate	729 (271)	2470 (473)	3393 (365)	6576 (416)	78 (36)	140 (51)	334 (47)	708 (81)

The numbers in parentheses represent the 95% confidence limits of the results.

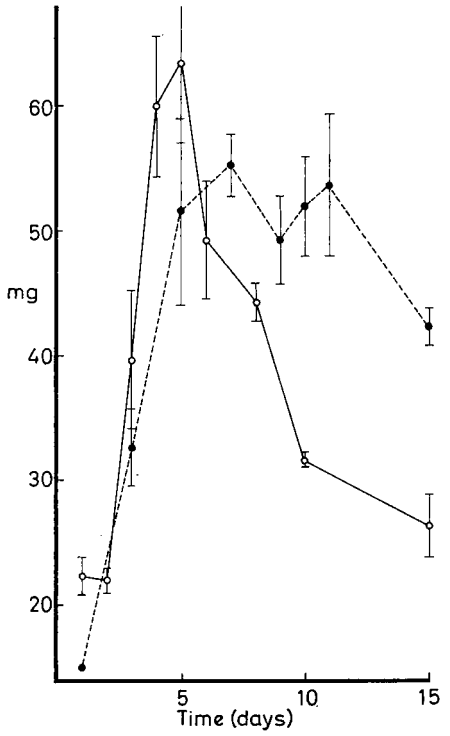

FIG. 1. Time-organ weight plots of androstanolone acetate and valerate on seminal vesicles (mg). Dose 1 mg. The limits represent ± one standard deviation. Mean control weight = 10·0 mg (σ = 1·7). —○— Treatment with androstanolone acetate. —●— Treatment with androstanolone valerate.

probability to around 50% $F_{9,20} = 0{\cdot}98$ $\alpha(0{\cdot}50) = 0{\cdot}96$. Similar results were obtained by the above mathematical treatment, with the androgenic activities of the nandrolone series and with both the androgenic and anabolic activities of the androstanolone esters.

As indicated earlier, the doses of esters employed in the present work represent the optimum range, and extrapolation of log dose-log cumulative weight plots outside it, is pointless, The small variations due to deviation from parallelism, which occur within the useful dose range are therefore probably not important and are indicated by the actual cumulative weights recorded. With this in mind, it was considered that an adequate representation of biological activity is given by the cumulative weight corresponding to a dose of 2 μM, the mid-point of the optimum range quoted above. These are recorded in Table 2 and it may be observed that androgenic activities of the nandrolone esters go through a maximum at the hexanoate, while anabolic activities increase irregularly as the homologous series is ascended. Anabolic-androgenic ratios for the nandrolone series are shown in Table 2, quoted relative to the propionate. While neither anabolic nor androgenic response varied uniformly as the homologous series was ascended, the anabolic-androgenic ratios form a smooth curve, passing through a minimum at the butyrate and then increasing. It is interesting to note that in this series, the decanoate, currently employed as an anabolic agent, possesses high values for both anabolic activity and anabolic-androgenic ratio. Cumulative weights obtained with the formate to valerate esters of androstanolone are given in Table 1. The derived anabolic-androgenic ratios (Table 2) relative to androstanolone valerate follow a similar sequence to the nandrolone series. Richards' results for testosterone esters (1972) also suggest a minimum in this region of the homologous series.

Anabolic and androgenic effects of steroid esters have been correlated with physical properties (Chaudry & James, 1974; James, Nicholls & Richards, 1975). The ester side-chain was considered to be a molecular appendage modifying the physical

Table 2. *Regression data and anabolic-androgenic (A/A) ratios for androstanolone and nandrolone esters.*

	Anabolic activity			Androgenic activity			
Ester	r[a]	Slope	Log response[b] (mg days)	r[a]	Slope	Log response[b] (mg days)	A/A ratio
Nandrolone series							
Formate	0·97	0·76	2·87 ± 0·12	0·87	1·06	2·23 ± 0·38	13
Acetate	0·97	1·04	2·99 ± 0·18	1·00	1·05	2·40 ± 0·06	11
Propionate	0·99	0·83	3·14 ± 0·11	0·99	0·57	2·61 ± 0·13	10
Butyrate	0·91	0·66	2·98 ± 0·28	1·00	1·24	2·59 ± 0·07	7
Valerate	0·99	0·79	3·31 ± 0·08	0·95	0·75	2·83 ± 0·18	9
Hexanoate	0·99	0·93	3·45 ± 0·61	1·00	1·17	2·97 ± 0·13	9
Heptanonate	0·97	1·21	3·65 ± 0·27	0·99	1·04	3·00 ± 0·16	13
Octanoate	1·00	1·18	3·64 ± 0·09	1·00	1·03	2·92 ± 0·08	15
Nonanoate	0·98	1·07	3·59 ± 0·19	0·98	1·06	2·78 ± 0·23	19
Decanoate	0·98	1·15	3·76 ± 0·22	0·99	1·38	2·82 ± 0·18	25
Undecanoate	0·99	1·51	3·71 ± 0·21	0·97	1·56	2·67 ± 0·41	32[d]
Androstanolone series							
Formate	0·95	0·50	2·90 ± 0·10	0·98	0·78	2·65 ± 0·11	9
Acetate	0·88	0·47	3·16 ± 0·16	0·98	0·69	2·88 ± 0·09	9
Propionate	0·98	0·60	3·09 ± 0·11	0·97	1·03	2·91 ± 0·24	7
Butyrate	0·98	0·60	3·21 ± 0·08	0·99	0·47	2·92 ± 0·04	9
Valerate	1·00	0·83	3·33 ± 0·04	0·98	0·72	2·99 ± 0·09	10

[a] Correlation coefficient; [b] Response to 2 μM dose ± confidence limits ($P' = 0{\cdot}05$); [c] Nandrolone series; nandrolone propionate = 10. Androstanolone series; androstanolone valerate = 10. [d] Estimated.

properties of the parent alcohol while the basic biological action was attributed to the alcohol, This latter hypothesis has been supported by the recent work of Solo, Bejba & others (1975). Changes in biological activity were linked to the influence of the physical properties of the ester on physiological processes, in particular the movement from the site of administration to the target site. The difference between the responses of levator anus and androgen indicator organs may be linked to this phenomenon, which Hansch, Muir & others (1963) have described as a 'random walk' across a series of hydrophilic and lipophilic barriers. Distribution coefficients become progressively more lipophilic as each methylene group is added in an homologous series and it is possible that the optimum distribution coefficient for the androgenic random walk is reached at nandrolone heptanoate, while the optimum for anabolic activity is somewhere in excess of that of the decanoate. Substantiation of this hypothesis would require detailed study of the pharmacokinetic profiles of the esters and parent alcohols with particular reference to sites of administration and target zones. Such information may also help explain the minimum value for anabolic-androgenic ratio of the steroid esters in the region of butyrate and propionate.

The results presented here indicate the value of the method employed for measuring anabolic and androgenic activities and provide experimental support for the use of nandrolone decanoate as an anabolic agent.

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