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INHIBITION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE BY STEROIDS—VIII: EFFECTS OF SYNTHETIC C_{19}- AND C_{20}-STEROIDS UPON PLACENTAL GLUCOSE-6-PHOSPHATE DEHYDROGENASE

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SUMMARY

Various synthetic C_{19}- and C_{20}-steroids or their derivatives, including a series of new compounds, were tested as inhibitors of human placental glucose-6-phosphate dehydrogenase. The alkyl esters of 5-etienic acid with a chain length of C_1–C_4 turned out to be quite effective inhibitors, whereas the free 5-etienic acid as well as its N-butyl amide lacked any inhibitory properties.

INTRODUCTION

The influence of steroids upon human glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49) has been discussed in numerous publications[1–5]. Although the inhibition of said enzyme activity usually is tested by *in vitro* techniques, also under physiological conditions a distinct inverse relationship seems to exist between G-6-PDH activity in erythrocytes and the levels of sulfoconjugated dehydroepiandrosterone (DHEA, 3β-hydroxy-5-androsten-17-one) as one of the most effective allosteric inhibitors of this enzyme [6–8]. Attempts to reduce high activities of human red blood cell G-6-PDH in psoriatics by administration of DHEA sulfate, however, remained unsuccessful due to increased reduction of the 17-oxo group[9]. Therefore, the search for other potential inhibitors of human G-6-PDH was continued. In the course of previous investigations a distinct inter-relationship could be established between the chemical structure and said physiological activity[4]. According to these conclusions a 17- or 20-oxo group and an equatorial 3β-hydroxy group in a planar ring A/B configuration present the structural features for optimal inhibitory activity. Since the rapid reduction of the 17-oxo group, experienced in psoriatics with their hyperactive 17β-hydroxy steroid oxidoreductase, had caused a complete loss of inhibitory activity, the replacement of the 17-oxo group by the carbonyl function of the 17β-carboxylic ester group should avoid such an inactivation and still provide the desired physiological activity.

In the present communication, therefore, the effects of 5-etienic acid (3β-hydroxy-5-androstene-17β-carboxylic acid) and its derivatives, as well as of hitherto untested C_{19}-steroids upon human placental G-6-PDH, are reported.

EXPERIMENTAL

Of the various compounds tested the ethyl, butyl and octyl ester, as well as the N-butyl amide of 5-etienic acid, were prepared as follows, while the other compounds were purchased from Steraloids (Inc., Pawling N.Y., U.S.A.).

Preparation of 5-etienic acid derivatives

1. *5-etienic acid ethyl ester.* To a solution of 0·3 mmol (96 mg) 5-etienic acid in 3 ml dioxane the diazoethane from 1 mmol (147 mg) 1-ethyl-1-nitroso-3-nitro-guanidine in 3 ml ether[10] was added during 15 min at room temperature. After additional 30 min at room temperature the solution was evaporated to dryness under vacuum and the ester isolated by preparative thin layer chromatography of the residue on silica gel G_{254} in chloroform–dioxane (94:6 v/v) (R_f = 0·65). Yields: 75 mg (77%); m.p.: 128–129°C; $(\alpha)_D^{22}$ = −24·5° (chloroform, c = 0·6); analysis: calculated for $C_{22}H_{34}O_3$ (346·5) 76·26% C, 9·88% H; found: 76·02, 9·59.

2. *5-etienic acid butyl ester.* From 0·3 mmol (96 mg) 5-etienic acid and 1·0 mmol (190 mg) 1-butyl-1-nitroso-3-nitro-guanidine as described under 1. Yields: 80 mg

(71%); m.p.: 150–151°C; $(\alpha)_D^{22} = -19{\cdot}1°$ (chloroform, c = 0·8); analysis: calculated for $C_{24}H_{38}O_3$ (374·6) 76·96% C, 10·23% H; found: 76·81, 10·02.

3. *5-etienic acid octyl ester.* From 0·3 mmol (96 mg) 5-etienic acid and 1·0 mmol (245 mg) 1-octyl-1-nitroso-3-nitro-guanidine as described under 1. Yields: 52 mg (40%); m.p.: 86–88°C; $(\alpha)_D^{22} = -22°$ (chloroform, c = 1·0); analysis: calculated for $C_{28}H_{46}O_3$ (430·7) 78·09% C, 10·77% H; found: 77·89, 10·45.

4. *5-etienic acid N-butyl amide.* 0·5 mmol (190 mg) 3β-acetoxy-5-etienic acid chloride[11] and 1·3 mmol (100 mg) n-butylamine in 5 ml benzene were stirred for 2 h at room temperature. After filtration from precipitated salt the filtrate was evaporated to dryness under vacuum and the residue boiled for 3 h in 5 ml 1·4% potassium carbonate in 60% methanol. After cooling and filtration, the filtrate was evaporated to dryness under vacuum and the residue chromatographed on silica gel G_{254} in chloroform–dioxane (94:6 v/v) ($R_F = 0{\cdot}45$). Yields: 140 mg (75%); m.p.: 191–192°C; $(\alpha)_D^{22} = -40{\cdot}4°$ (chloroform, c = 1·2); analysis: calculated for $C_{24}H_{38}NO_2$ (373·6) 77·16% C, 10·52% H, 3·75% N; found: 77·20, 10·30, 3·40.

In the I.R. spectra of the esters 1–3 distinct absorption bands at 1705/cm. and 3160/cm. were registered, indicative of the carboxyl and the hydroxy group at C-17 and C-3 respectively. The I.R. spectrum of the N-butyl amide exhibited strong absorption bands at 1535/cm., 1625/cm., as well as a broader band at 3200/cm., reflecting the presence of the amide group and the -OH and -NH- group respectively.

Furthermore, in the sulfuric acid ethanol absorption spectra of all derivatives a pronounced absorption maximum at 407–408 nm was observed, which is characteristic of 3β-hydroxy-5-ene-steroids.

G-6-PDH inhibition tests

Human placental G-6-PDH was purified as outlined in a previous communication[12]. The K_m-value of the 112–146-fold enriched enzyme (specific activity = 1 780 – 2 330 mU/mg protein) ranged between 1·15 and 1·28 × 10^{-4} M for G-6-P and 0·96 and 1·05 × 10^{-4} M for NADP. The inhibition tests were performed with 0·1 ml of the enzyme preparation, 3·0 ml 0·05 M triethanolamine/0·005 M EDTA buffer of pH 7·6, 0·1 ml 0·01 M NADP solution, and 0·02 ml dioxane, containing steroid or steroid derivative. The final concentration of the particular compound corresponded to a 10^{-5} M solution. After addition of varying amounts of G-6-P in 0·05 ml water, changes in the absorption at 366 nm were registered over 5 min at 25°C and compared to those of appropriate blanks. For evaluation of the results the method of Hunter and Downs[13] was employed.

RESULTS AND DISCUSSION

The inhibition of purified human placental G-6-PDH by hitherto untested C_{19}- and C_{20}-steroids or their derivatives is demonstrated by the Hunter and Downs diagrams in Fig. 1, as well as their corresponding K_i-values in Table 1. From these figures it becomes apparent that the alkyl esters of 5-etienic acid represent effective inhibitors of human G-6-PDH, the inhibition being of the allosteric type. Whereas 5-etienic acid itself lacked any inhibitory activity, the methyl ester already exhibited a K_i-value lower than that of DHEA. Prolongation of the chain length of the esterified alkanol to C_2 and C_4 resulted in a further decrease of K_i-values from 0·57 × 10^{-5} M, to 0·51 × 10^{-5} M and 0·48 × 10^{-5} M. The octyl ester, however, turned out to be considerably less active in the enzyme inhibition test. The N-butyl amide, like the free 5-etienic acid, failed to exert inhibitory effects upon the placental G-6-PDH. The other C_{19}- or C_{20}-steroids were found to inhibit G-6-PDH to a minor degree. As it appears, an esterified 17β-carboxyl group and a 17-oxo or 20-oxo group alike provide an optimal structure in ring D of the steroid molecule for its attachment to the enzyme protein, presumably by hydrogen bonding to the carbonyl oxygen of such steroids.

In the course of the present investigations, however, several preparations of purified placental G-6-PDH

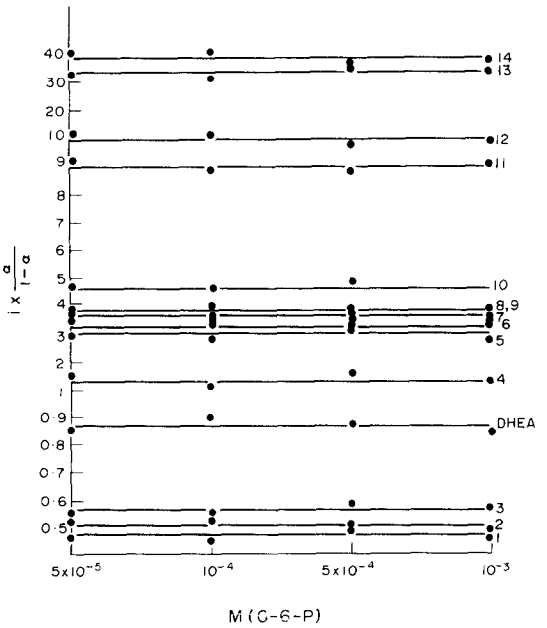

Fig. 1. Hunter-Downs diagram for compounds 1–14 (see Table 1): $\alpha = v_i/v$; v = velocity; v_i = velocity in presence of inhibitor; i = concentration of inhibitor in mol/l; (G-6-P) = concentration of glucose-6-phosphate in mol/l.

Table 1. Inhibition of human placental G-6-PDH by C_{19}- and C_{20}-steroids or their derivatives

	Steroid	K_i-value for G-6-P
1	3β-hydroxy-5-androsten-17β-carboxylic acid butyl ester	0.48×10^{-5} M
2	3β-hydroxy-5-androsten-17β-carboxylic acid ethyl ester	0·51
3	3β-hydroxy-5-androsten-17β-carboxylic acid methyl ester	0·57
4	3β-hydroxy-5-androsten-17β-carboxylic acid octyl ester	1·3
5	17β-amino-5-androsten-3β-ol	3·1
6	5-androstene-3β,17β-diol, 17β-acetate	3·2
7	3β-hydroxy-5-androsten-16-one	3·6
8	3β-hydroxy-5α-androstane-17β-carboxylic acid methyl ester	3·8
9	3β-hydroxy-5α-androstan-16-one	3·8
10	3β,17β-dihydroxy-5-androsten-16-one	4·6
11	5-androsten-3β,17β-diol, 17β-propionate	9·0
12	17α-cyano-5,16-androstadien-3β-ol	10
13	3β-hydroxy-5-androstene-17β-carboxylic acid N-butyl amide	33
14	3β-hydroxy-5-androstene-17β-carboxylic acid	38
	3β-hydroxy-5-androsten-17-one (DHEA)	0·87

showed an unexpected, weak response to either of the listed 5-etienic acid alkyl esters. Similar findings were obtained with G-6-PDH preparations from guinea-pig liver, where the inhibition by 10^{-5} M concentrations of 5-etienic acid alkyl esters varied between 9 and 54%. The apparent inefficiency of these compounds in such enzyme preparations was ascribed to an extensive hydrolysis of the carboxylic acid esters during incubation. Indeed, the analysis of the particular incubates revealed that up to 60% of isolated 3β-hydroxy-5-ene-C_{20}-steroids occurred in the fraction of free acidic steroids and consisted predominantly of free 5-etienic acid, which was found to be completely inactive in the G-6-PDH inhibition test. When linoleic acid methyl ester was added to such incubates in 10^{-5} M concentrations, the inhibition of purified placental G-6-PDH by equimolar concentration of 5-etienic acid methyl ester increased from 8–11% to 12–17%, hinting at a competitive inhibition of suspected esterase activity. Furthermore, after i.v. administration of 7α-^{3}H-5-etienic acid methyl ester in a normal female volunteer an immediate and substantial hydrolysis of the substrate was observed[14]. As to be reported in a forthcoming communication also the incubation of human erythrocytes with 7α-^{3}H-5-etienic acid ^{14}C-methyl ester resulted in an almost complete hydrolysis of the substrate. Since aryl esterase II, abundant in human blood and tissues, is known to be rather unspecific and actually represents a carboxyl esterase (EC 3.1.1.1) [15, 16], this enzyme may very well be responsible for the hydrolysis of the alkyl ester. It appears that only a careful purification of enzyme preparations by fractionate ammonium sulfate precipitation and repeated adsorption unto calcium phosphate gel may remove the contaminating esterase activity. The K_i-values in Table 1 were gained with a G-6-PDH preparation, which seemed to be free of esterase activity, thus allowing a comparative evaluation.

Concerning the therapeutic application of 5-etienic acid alkyl esters, the aforementioned inactivation by cleavage of the ester bond under *in vitro* as well as *in vivo* conditions obviously precludes their envisioned oral or systemic administration to patients with such metabolic diseases as psoriasis or hyperlipoproteinemia, associated with a G-6-PDH hyperactivity[17]. Interestingly enough, however, preliminary experiments with 5-etienic acid methyl ester and other G-6-PDH inhibitors like DHEA, DHEA sulfatide (dipalmitoyl glycerosulfate), and DHEA lauryl sulfate showed that in cultures from neoplastic human tissues the cytostatic activity of the former compound at least could be compared to that of DHEA or its derivatives [18]. At the same time, these findings support the conclusion that 5-etienic acid methyl ester just like DHEA or its derivatives may exert certain biological effects by suppression of G-6-PDH activity.

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