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CELLS FROM RAT MARROW: SENSITIVITY TO EFFECTS OF CERTAIN PENTACYCLIC TRITERPENOIDS*

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Abstract—Numerous pentacyclic triterpenoids were shown to inhibit protein biosynthesis in the cells of rat marrow. The magnitude of the effect depends on the chemical structure of the triterpenoids. Hederagenin glycosides with one carbohydrate chain at 0–3 possess the highest activity. Additional presence of a second carbohydrate chain at C-28 or of a hydroxyl group at C-16 generally reduces glycoside activity. Rat marrow cells differ in degree of sensitivity from the action of triterpene glycosides. Cells of the erythroid series proved to be most sensitive.

Pentacyclic triterpenoids are widespread in the plant kingdom [1–5]. Many of these compounds are physiologically active and possess a broad spectrum of medical and biological effects [1, 2, 6–10]. In the rat marrow cells, certain triterpenoids inhibit the incorporation of [^{14}C]alanine in the acid-insoluble fraction [11]. This effect was used in the present work to study the relationship between the chemical structure and biological activity of some pentacyclic triterpenoids.

MATERIALS AND METHODS

The rat marrow cell suspension was prepared from tibia and femur bones [12]. Incubation was performed in the medium consisting of saline (NaCl, 95 mM, KCl, 5 mM, $MgCl_2 \cdot 6H_2O$, 7,4 mM, tris buffer (PH 7,6) 10 mM; $NaHCO_3$, 2 mM) and 5,5 mM of glucose. The relationship between the chemical structure and physiological activity of triterpenoids was examined by incubating the marrow cells in test tubes for 3 hr at 37°, in a drum, at the rotation speed of 720 rev/min [13]. The incubation mixture consisted of a 1 ml cell suspension (about 40×10^6), a 0.04 ml aqueous [^{14}C]alanine solution with a total activity of 0.4 μCi and a 0.1 ml water–alcohol solution of the substances studied. Ethanol concentration in the incubation medium did not exceed 0.5%.

After incubation, the reaction was terminated by adding an equal volume of a 10% trichloracetic acid (TCA). The residue was smeared onto membrane filters and washed with 100 ml of 5% TCA. Radioactivity was determined in an end-type counter.

In some tests, the marrow cells were incubated for 60 min in a buffer solution previously suggested for cell isolation [12]. Following incubation, the cells were washed three times with a cold buffer, layered onto a saccharose gradient (1.4–2.0 M) and centrifuged at 4,000 rev/min for 30 min at 4° on a "K-23" centrifuge. Fractions (1 ml) were collected from the gradient to count the number of cells. The cells of each fraction were diluted with the medium up to 10 ml and thoroughly mixed. Optical density of the suspension was measured on a photoelectrocalorimeter. $\lambda = 540$ nm. It was preliminarily established that 1 o.D. of the cell suspension was equal to 20×10^6 cells estimated in Goryaev chamber. The protein content was determined by the method of Lowry *et al.* [14]. The ultra-violet and visible spectra were recorded on a "Specord" instrument after precipitating the cells.

Compounds I–XVI and XVII–XXII were obtained from the roots of the relict Far-Eastern *Caulophyllum robustum* M. [15] and *Platycodon grandiflorus* [16, 17], respectively.

RESULTS

As is apparent from Table 1, caulosides A (VII), B (XIV) and C (VIII) inhibit by 50 per cent the incorporation of [^{14}C]alanine in the acid-insoluble fraction of rat marrow cells maintained at concentrations of 5, 70, and 7 μg/ml, respectively. Caulosides D (IX) and E (X) in concentrations of up to 100 μg/ml lack this effect. The progenin of cauloside E (XI) inhibits incorporation of [^{14}C]alanine by 50 per cent (conc. 100 μg/ml).

Compared with the effects of caulosides A (VII) and C (VIII), those of hederagenin (I) and its derivatives (II–VI) result in considerably less inhibition of [^{14}C]alanine incorporation in the acid-insoluble fraction of rat marrow cells; in this case, complete inhibition is not attained. The acetate of cauloside C (XII) also reveals less activity than cauloside C (VIII).

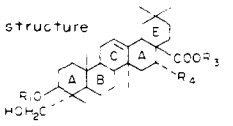
A somewhat different picture (Table 1) is observed when examining the inhibiting effect of cauloside B (XIV), its genin (XIII) and derivatives (XV–XVI): the genin acetonide of cauloside B (XV) shows higher activity than cauloside B (XIV).

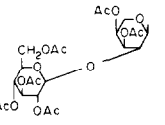
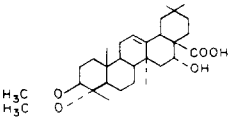
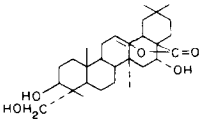
Among the triterpenoids from *Platycodon grandiflorus*, platycodoside C (XVIII) in concentrations of 25 μg/ml inhibits incorporation of [^{14}C]alanine in the acid-insoluble fraction of rat marrow cells by 93 per cent and possesses the highest activity (Fig. 1). As for other compounds from this plant, they possess considerably less activity (XVII, XIX–XXII).

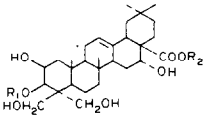
* Translated by Joseph C. Shapiro.

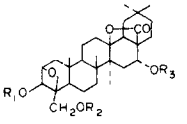
Table 1

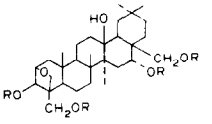
General structure



No	Compound	R_1	R_2	R_3	R_4	Concn. μg/ml for 50% inhibition
I	Hederagenin	H	H	H	H	20
II	Hederagenin diacetate	Ac	Ac	H	H	35
III	Hederagenin methylester	H	H	CH_3	H	>100
Iy	Hederagenin 3'23_diacetate methyl ester	Ac	Ac	CH_3	H	>100
y	23-O-methylhederagenin methyl ester	H	CH_3	CH_3	H	60
yI	Acetonide of hederagenin	R_1-, R_2- = $C(CH_3)_2$		H	H	>100
yII	Cauloside A	L-Ara $\xrightarrow{\alpha}$	H	H	H	5
yIII	Cauloside C	D-Glc I $\xrightarrow{\beta}$ 2L-Ara $\xrightarrow{\alpha}$	H	H	H	7
IX	Cauloside D	L-Ara $\xrightarrow{\alpha}$	H	-Glc-Glc-Rha	H	>100
X	Cauloside E	D-Glc I $\xrightarrow{\beta}$ 2L-Ara $\xrightarrow{\alpha}$	H	-Glc-Glc-Rha	H	>100
XI	Cauloside E progenin V	H	H	-Glc	H	100
XII	Cauloside C acetate		Ac	H	H	20
XIII	Caulophyllogenin	H	H	H	OH	75
XIV	Cauloside B	L-Ara $\xrightarrow{\alpha}$	H	H	OH	70
XV	Acetonide of caulophyllogenin					10
XVI	Caullofillogenin γ-lacton					>100







XyII Platicodigenin $R_1=R_2=H$

XyIII Platicodoside C
R_1=Glc, R_2=Ara—Rha—Xyl—Glc

XIX Platicodogenin $R_1=R_2=R_3=H$

XX Acetonide of platicodogenin
$R_1=R_2=C(CH_3)_2$, $R_3=H$

XXI Pentaol R=H

XXII Pentaol acetate R=Ac

With increased concentrations of cauloside B (XIV) in the incubation mixture, those substances that possess maximum absorption at 272 and 414 nm and are precipitated by a 10% perchloric acid pass from the cells to the mixture. With cauloside B (XIV) concentrations of 30 and 50 μg/ml, 50 and 100 per cent of the substances, respectively, are observed in the mixture. In this case, [^{14}C]alanine incorporation in the cell acid-insoluble fraction is inhibited by 15 and 44 per cent, respectively (Fig. 2).

The results of our investigations showed that centrifugation of suspensions of rat marrow cells cause the formation of three cell zones in the linear gradient of saccharose density. The cells in zone III (upper zone) decompose when cauloside B (XIV) acts on this suspension (Fig. 3).

DISCUSSION

One of the important problems in the study of triterpenoids is the investigation of the relationship between the chemical structure and physiological activity of these compounds. With respect to this, it should be noted that caulosides D (IX) and E (X) differ from caulosides A (VII) and C (VIII) in the presence of an additional carbohydrate chain at C-28;

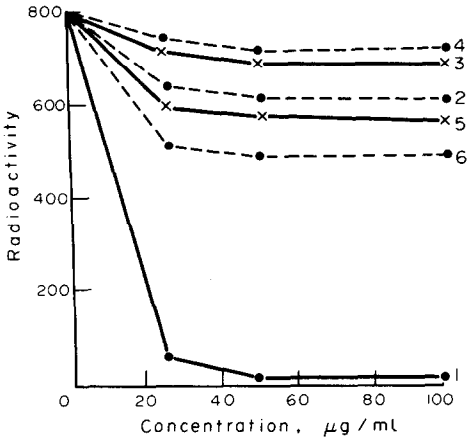


Fig. 1. Effects of 1-platycodoside C (XVIII), 2-platycodigenin (XVII), 3-platycodogenin acetonide (XX), 4-platycodogenin (XIX), 5-reduced platycodoside (XXI) and 6-pentaol acetate (XXII) on incorporation of [^{14}C]alanine in acid-insoluble fraction of rat marrow cells (experimental conditions are described in Methods Radioactivity is expressed in counts/100 sec. Average values for three membrane filters.

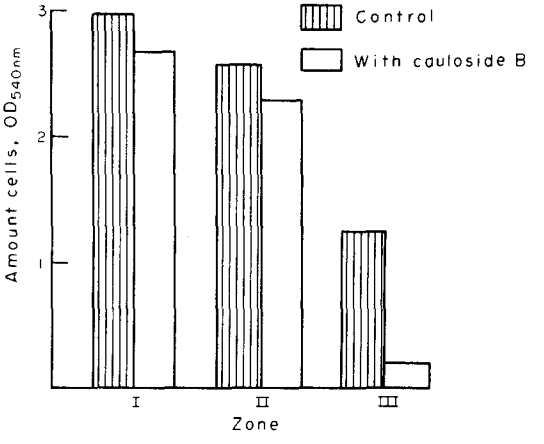


Fig. 3. Effect of cauloside B (XIV), conc. 50 μg/ml, on distribution of suspension of rat marrow cells in saccharose gradient. Experimental conditions are described in Methods.

at the same time, these glycosides show no inhibiting effect for the concentrations tested (Table 1). A similar regularity is observed in the study of antimicrobial and cytotoxic activities of these compounds. [9, 18]. Triterpenoid glycosides containing hederagenin [15] or oleanolic acid [19] as aglycons and incorporating two carbohydrate chains at C-3 and C-27 or C-3 and C-28 generally show very low physiological activity [20, 21].

Like caulosides D (IX) and E (X), platicodoside C (XVIII) also has two carbohydrate chains C-3 and C-28. However, unlike caulosides, it shows high physiological activity (Fig. 1). This property of platicodoside C (XVIII) is probably caused by the specific

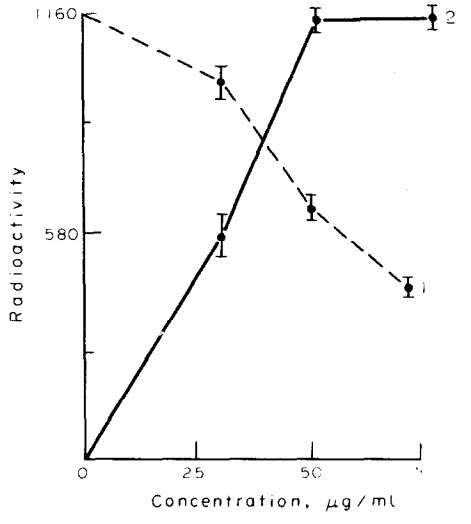


Fig. 2. Effect of cauloside B (XIV) on incorporation of [^{14}C]alanine in acid-insoluble fraction of rat marrow cells (I) and hemoglobin content in culture medium (2). Radioactivity is expressed in counts/100 sec. Average values for 3 membrane filters.

structure of the aglycon, which differs from hederagenin with respect to the presence of additional functional groups [16, 17]. Besides, substitution of carbohydrate chains in platicodoside C (XVIII), in C-3 and C-28, for hydrogen (XVII) leads to a considerable lowering of activity (Fig. 1).

Cauloside B (XIV) is almost ten times weaker in activity than cauloside A (VII), and it differs from cauloside A (VII) in the presence of hydroxyl group at C-16 (α). The results of these studies are in agreement with previously published data [9, 18]. Hence, the hydroxyl group at C-16 considerably reduces the physiological activity of the glycoside.

In all the cases studied, the glycosides reveal higher activity than their aglycons and acetates. Most of the genin derivatives, with the exception of the genin acetonide of cauloside B (XV), show lower activity than the starting glycosides. The inhibiting effect of these derivatives attains a definite value and remains on the same level despite increased concentration of those substances. This is possibly associated with the fact that not all marrow cells, but only their individual populations, are sensitive to the action of genin derivatives.

The literature shows that the rat marrow cell suspension consists of a heterogeneous cell population, which includes cells of the erythroid series [22]. Saponins, in turn, possess high haemolytic activity, i.e., they cause lysis of erythrocytes with subsequent release of hemoglobin [6]. Based upon this fact, we thought it would be interesting to separate the above suspension into several fractions in order to study cell sensitivities in individual fractions to the effect of cauloside B (XIV). We have shown that, as a result of centrifugation in a saccharose density gradient, rat marrow cells are distributed over three zones. Zone III is enriched by cells of the erythroid series, since they are destroyed by ammonium chloride [23]. Joint incubation of a cell suspension with cauloside B (XIV) for 60 min leads to cell decomposition in zone III (Fig. 3); as a result, the hemoglobin (Fig. 2) containing [^{14}C]alanine appears in the culture medium. Hence, the inhibiting effect of cauloside B (XIV) is to some extent caused by the lysis of cells of the erythroid

series. With increased concentrations of the glycoside in the cells of the leucocytary series in zones I and II, protein biosynthesis is suppressed, though without noticeable cell decomposition.

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