quefied nutrient agar[2]. After hardening, the plates were inoculated with an aqueous suspension of the test organism, incubated at 34°, and examined after 48 hr.

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COMMUNICATIONS

Antiviral Activity of Triterpenoid Saponins Containing Acylated β-Amyrin Aglycones

Keyphrases □ Triterpenoid saponins containing acylated β-amyrin aglycone structure—antiviral activity □ Antiviral activity—11 triterpenoid saponins containing acylated β-amyrin aglycone, structure evaluated

Sir:

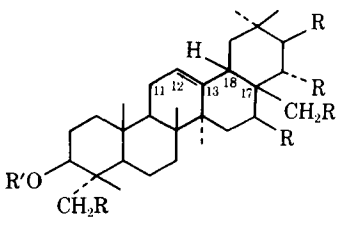
Previously we reported (1) the *in vitro* antiviral activity of gymnemic acids A and B, which were isolated from *Gymnema sylvestre* R. Br. leaves and were shown to be acylated derivatives of β-amyrin glycosides (2–5). Gymnemic acid A also exhibited significant antiviral activity against influenza A virus in mice when administered intraperitoneally (1). The wide occurrence in nature (6) of the triterpenoid saponins containing the β-amyrin skeleton (I) prompted us to evaluate the antiviral activity of this group of natural products. The present communication reports the *in vitro* anti-influenzal activity of 11 naturally occurring triterpenoid saponins structurally related to the gymnemic acids. These results, together with those obtained with gymnemic acid derivatives, permit delineation of preliminary structure–antiviral activity relationships among the triterpenoid saponins studied.

Tables I and II summarize the *in vitro* antiviral activity of gymnemic acids, their derivatives, and various triterpenoid saponins against influenza (A_2/Japan 305) virus. The antiviral activity was evaluated by measuring either the decrease in the 48-hr infectivity yield of virus in eggs from calf kidney cells inoculated with virus (1) or the inhibition of hemadsorption of guinea pig erythrocytes by virus on primary calf kidney cells (15). Based upon the preliminary antiviral results obtained with gymnemic acids (II–V), their derivatives (VI–IX), and other structurally related triterpenoid saponins (X–XX), the following remarks may be made regarding the structure–antiviral activity relationships among the triterpenoid saponins containing β-amyrin skeleton:

1. The majority of the triterpenoid saponins containing the acylated β-amyrin skeleton employed in this study exhibited anti-influenzal activity *in vitro*.

2. The presence of aldehyde (XI, XIV, and XVI) and/or carboxyl (XII, XV, XVI, and XIX) groups in place of or in addition to acyl groups in the β-amyrin skeleton of triterpenoid saponins markedly reduced their antiviral activity.

3. Alteration of the basic β-amyrin skeleton (I), *e.g.*, replacement of the 12,13-double bond with 11,13(18)-diene as in saikosaponin b (XVIII), completely abolished *in vitro* anti-influenzal activity. However, the activity is restored if the 11,12-double bond and methyleneoxy group at the 13,17-position



I: R = R′ = H
VII: R = OH, R′ = H
IX: R = $OCOCH_3$, R′ = $COCH_3$

Table I—Antiviral Activity of Gymnemic Acids and Their Derivatives against Influenza (A_2/Japan 305) Virus *In Vitro*

Compound Number	Compound	Reference	Infectivity[a] Concentration, μg/ml Ethanol	Infectivity[a] Treated	Infectivity[a] Control	Activity
II	Gymnemic acid A	2	200	1	5.5	Good
III	Gymnemic acid B	2	200	4.3	7.3	Moderate
IV	Gymnemic acid C	2	200	7.0	7.3	None
V	Gymnemic acid D	2	200	7.5	7.3	None
VI	Gymnemic acid A methyl ester	7	200	1	4.5	Good
VII	Gymnemagenin	7–9	200	4.5	4.3	None
VIII	Genin mixture[b]	3	200	1	5.5	Good
IX	Gymnemagenin acetate mixture[c]	7	100[d]	4.0	4.3	None

[a] This represents the ability of compounds tested to decrease the 48-hr infectivity yield of virus (reported as $\log_{10}$ of the infectious dose) in eggs from primary calf kidney cells inoculated with virus. See Ref. 1 for experimental details. [b] The genin mixture was obtained by acid hydrolysis of the crude gymnemic acid mixture and contains no sugar residues (3). [c] Acetylation of gymnemagenin gives rise to a mixture of acetate derivatives, the hexaacetate IX being the major product (7). [d] Maximum tolerated concentration.

Table II—Antiviral Activity of Triterpenoid Saponins against Influenza (A_2/Japan 305) Virus *In Vitro*

Compound Number	Compound	Reference	Plant Source	Concentration[a], μg/ml Ethanol	Maximum Tolerated Concentration, μg/ml	Inhibition[b], %
X	Aescin[c]	10	*Aesculus hippocastanum* L. (Hippocastanaceae)	12.5	12.5	92
XI	Cyclamin A	10	*Cyclamen europeum* (Primulaceae)	12.5	12.5	33
XII	Glycyrrhizin[c]	10	*Glycyrrhiza glabra* L. (Leguminosae)	100[d]	—	37
XIII	Hederocoside C	10	*Hedera helix* L. (Araliaceae)	100[d]	—	54
XIV	Primula saponin[c]	10	*Primula veris* L. (Primulaceae)	6.2	6.2	89
XV	Prosapogenin	11	*Polygala senega* L. (Polygalaceae)	100[d]	—	36
XVI	Quillaja saponin[c]	10	*Quillaja saponica* (Rosaceae)	12.5	25[e]	22
XVII	Saikosaponin a	12	*Bupleurum falcatum* L. (Umbilliferae)	50[d]	—	69
XVIII	Saikosaponin b	12	*Bupleurum falcatum* L. (Umbilliferae)	100[d]	—	0
XIX	Senegin	10	*Polygala senega* L. (Polygalaceae)	12.5	50[e]	34
XX	Theasaponin[c]	10	*Thea sinensis* L. (Theaceae)	6.2	6.2	73
II	Gymnemic acid A	2	*Gymnema sylvestre* R. Br. (Asclepiadaceae)	100	500[e]	63
VII	Gymnemagenin	7–9	*Gymnema sylvestre* R. Br. (Asclepiadaceae)	50[d]	—	0
IX	Gymnemagenin Acetate[f]	7	*Gymnema sylvestre* R. Br. (Asclepiadaceae)	100[d]	—	0
XXI	Amantadine[g]	13, 14	—	25	100	98

[a] The concentrations tested were at 100 μg/ml or at the maximum tolerated concentration if availability of samples permitted. [b] Inhibition of quantitative hemadsorption of guinea pig erythrocytes by virus on primary calf kidney cells. See Ref. 15 for experimental details. [c] Crude sample. [d] Maximum concentration tested. [e] Estimated maximum tolerated concentration. [f] Acetylation of gymnemagenin gives rise to a mixture of acetate derivatives, the hexaacetate IX being the major product (7). [g] Synthetic compound (16, 17) used as a positive control.

are present in place of the 12,13-double bond, as in saikosaponin a (XVII).

4. For *in vitro* activity, a sugar residue in the triterpenoid compounds tested does not appear to be essential since sugar-free acylated derivatives of gymnemic acids (VIII) exhibited comparable antiviral activity. Also, methylation of the glucuronic acid moiety in gymnemic acid A (VI) did not produce any significant change in *in vitro* activity against influenza virus.

5. Polyhydroxy and polyacetyl derivatives of β-amyrin, *e.g.*, gymnemagenin (VII) and gymnemagenin acetate (IX), were found to be inactive as inhibitors of influenza virus *in vitro*.

Although a number of triterpenoid saponins are known to possess antimicrobial (10) and antiparasitic (6) activities, antiviral activity has not been previously indicated for this group of natural products. Gymnemic acid A appears to be the first triterpenoid saponin reported to exhibit antiviral activity both *in vitro* and *in vivo* (1).

However, the antiviral activity of various synthetic

steroids (18) and that of antibacterial agents, fusidic acid, cephalosporin P_1, helvolic acid, and related compounds (19,20) have been reported. Cardiac glycosides[1] (21–24) and steroidal glycosides isolated from starfish (25) also possess antiviral activity. Furthermore, the triterpenoid saponins (X–XIV, XVI, XIX, and XX) found to inhibit influenza virus in the present study were reported to be antibacterial agents (10).

At least one site of action of gymnemic acid A against influenza virus is indicated to be associated with relatively early events in the virus infectious cycle which may involve inhibition of virus–host cell attachment (1). Cardiac glycosides are believed to exhibit antiviral action by competing with infectious viruses for the virus-specific receptor sites on the cell membrane and adenosine triphosphatase molecules involved in virus–host cell interactions (24). The recent observation that gymnemic acid also inhibits adenosine triphosphatase activity (26) suggests that the mechanism of antiviral action of gymnemic acid may be similar to that proposed for cardiac glycosides.

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[1] In our *in vitro* tests, digitonin exhibited 100% inhibitory activity against influenza virus at a concentration of 12.5 μg/ml.

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Trapa bispinosa Starch as a Tablet Disintegrant

Keyphrases □ *Trapa bispinosa* starch—compared to wheat, potato, and maize starch as a possible tablet disintegrant □ Tablet disintegrants—trapa starch compared to wheat, potato, and maize starch □ Disintegrants, tablet—trapa starch compared to wheat, potato, and maize starch

Sir:

Trapa bispinosa (Faln. onagraceae) (1), commonly known as water caltrop or water chestnut, is an aquatic plant which has been grown in India since ancient times at negligible cost. It is widely used as a general article of food and has a palatable taste. It has been reported that the plant nut contains 73% starch. These preliminary data prompted us to study this starch[1] as a tablet disintegrant vis-à-vis other starches currently used.

Starch was isolated by the commonly used water extraction method. Preliminary physical and chemical analysis of trapa starch showed similar characteristics to those of maize starch, commonly used as a disintegrant for compressed tablets. Trapa starch passes all the tests described under the starch mono-

[1] Dr. V. K. Deshmukh, Professor of Pharmacognosy, Department of Pharmaceutical Sciences, Nagpur University, Nagpur, India, identified the plant *Trapa bispinosa* and the voucher specimen is deposited in the laboratory of the Department of Pharmaceutical Sciences, Nagpur University, Nagpur, India.