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BIOLOGICAL ACTIVITY OF STEROL GLYCOSIDES*

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Abstract—Reversal of the inhibition of the growth of *Tetrahymena pyriformis* W. by the analog, 6-methylpurine, can be obtained either with large amounts of adenine or with smaller amounts of a fraction obtained from saponified lecithin of soybeans. The active material has properties which indicate its identification as a β-D-glycoside of β-sitosterol. The presence of sugars other than glucose in the glycone moiety is also indicated. Synthetic β-sitosterol-β-D-glucoside has growth-promoting properties approximately equal to those of the isolated material; both materials are more active than free β-sitosterol.

IN AN investigation of the inhibitory effect of 6-methylpurine (6-MeP) on the growth of *Tetrahymena pyriformis*, it was found that sterols, phospholipids, peptides and branched chain fatty acids are effective in partially reversing the inhibition.[1] In the presence of these compounds, small amounts of adenine are active, but not in their absence; however, sufficiently large amounts of adenine alone (from twenty five to fifty times the quantity of the analog) are completely effective.

Various types of phospholipid-containing material were fractionated in order to determine which were the active substances. Glycolipids, inositol phosphatides, and phosphatidyl ethanolamine were all found to possess activity when tested in the presence of sterol, but not in its absence, whereas sterols are active without other addition.[1]

Soy bean lecithin, which has activity in the absence of sterols, was subjected to alkaline hydrolysis and separated into four fractions, non-saponifiable, fatty acid, water-soluble and an interfacial layer formed when the alkaline solution was extracted with ether. The fluffy interfacial material, insoluble in both ether and the aqueous phase, possessed greater activity at lower concentrations than any other single substance so far tested, including adenine. Further investigations were undertaken to determine the nature of this material.

MATERIALS AND METHODS

Tetrahymena pyriformis was grown in Medium A[2]. Adenylic acid and acetate were omitted from this medium and 6-methylpurine was added at a concentration of 2 μg/ml; various compounds or fractions of natural materials then were added to test for release of inhibition.

Commercial soy bean lecithin (SBL) was saponified with NaOH and extracted with ethyl ether to remove the non-saponifiable material (N). After acidification the

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extraction with ether was repeated to remove fatty acids (SI). At this point the interfacial material was removed as a separate fraction (SII). The remaining aqueous phase was neutralized and reduced in volume to remove ether (R). The ether was allowed to evaporate from the other fractions before testing.

Fraction SII was subjected to hydrolysis in ethanol containing a few drops of HCl.[3] After dilution with water and extraction with ether, the ether-soluble material was recrystallized five times from 85% ethanol. The aqueous residue was reduced in volume, borate added, and chromatographed on a Dowex-1 borate ion-exchange column.[4]

Fractions obtained from the column were assayed by the anthrone reaction for hexose and the orcinol reaction for pentose. Further identification was attempted by the use of the carbazole reaction, cysteine-sulfuric acid reaction for deoxyhexose and the resorcinol reaction for ketohexoses.[5] The anthrone reaction was modified by heating separate samples for 1, 2, 5, 10, and 15 min, respectively, since aldehexoses require more prolonged heating for development of full color (5–10 min) than do ketohexoses (2 min), deoxyhexoses (2–5 min) or pentoses. The pentose color rapidly disappears when heating is prolonged beyond 2 min.

RESULTS

As shown in Table 1, only crude SBL and fraction SII had appreciable activity alone. Neither the fatty acids nor the non-saponifiable materials was effective. In the

TABLE 1. RELEASE OF INHIBITION OF *Tetrahymena* BY 6-METHYLPURINE USING FRACTIONS FROM SAPONIFIED SOY BEAN LECITHIN

Additions (μg/ml)	Stigmasterol 0 (% optimal growth*)	Stigmasterol 50 (% optimal growth*)	Additions (μg/ml)	Stigmasterol 0 (% optimal growth)	Stigmasterol 50 (% optimal growth)
None	12	54	SBL 200	100	116
SII 1	76	—	N 50	22	75
2	89	—	N 100	29	95
5	97	—	SI 50	15	99
10	96	100	SI 100	13	100
50	95	100	R 50	11	72
100	100	100	R 100	8	80

Medium contains 6-MeP, 2 μg/ml.
* Optimal growth in presence of sterol glycoside = O.D. 0·250.

presence of stigmasterol, which gives partial reversal alone, the fatty acid fraction showed an effect. Investigation of SII indicated that it is probably identical with a sterol glycoside. The material is soluble in pyridine or hot glacial acetic acid. The Liebermann–Burchard reaction is positive. It melts with decomposition above 290 °C. The acetate melts at 168–169 °C (167–168·5 °C[3]; 166–167 °C[6]) and the benzoate at 198 °C (198 °C[6]). After acid hydrolysis[3] and extraction of the hydrolysis mixture with ether, the ether-soluble material, recrystallized five times from 85% ethanol, melted at 136 °C (136 °C[7]; 138·5 °C[8]; 137 °C[9] for β-sitosterol).

Chromatography of the water-soluble material (after ether extraction of the hydrolysate) in the borate system[3] gave several peaks with evidence of incomplete separation of a number of sugars. Use of the anthrone reaction, orcinol reaction, and

the cysteine–sulfuric acid reaction for methylpentoses (deoxyhexoses) indicated that a major peak consists of glucose and the others of mixtures of hexose, deoxyhexose and pentoses. One peak showed a positive reaction for a ketohexose.

Since chromatography of SII (without hydrolysis) on glass-fiber filter paper using benzene–ethanol as a solvent gave two spots with a positive sterol reaction, it is not possible to say whether the crude material consists of several β-sitosterol glycosides differing in the glycone moiety or whether the glycone may consist of a polysaccharide or a variety of polysaccharides.

Synthesis of β-sitosterol-β-D-glucoside[3, 8] showed that it possesses activity approximately equal to that of the isolated material and that both surpass the free sterol in activity. The β-D-glucosides of cholesterol and stigmasterol were also prepared and tested. Fig. 1 shows that all three synthetic glucosides were similar in activity to the natural material, stigmasterol-β-D-glucoside being the least active.

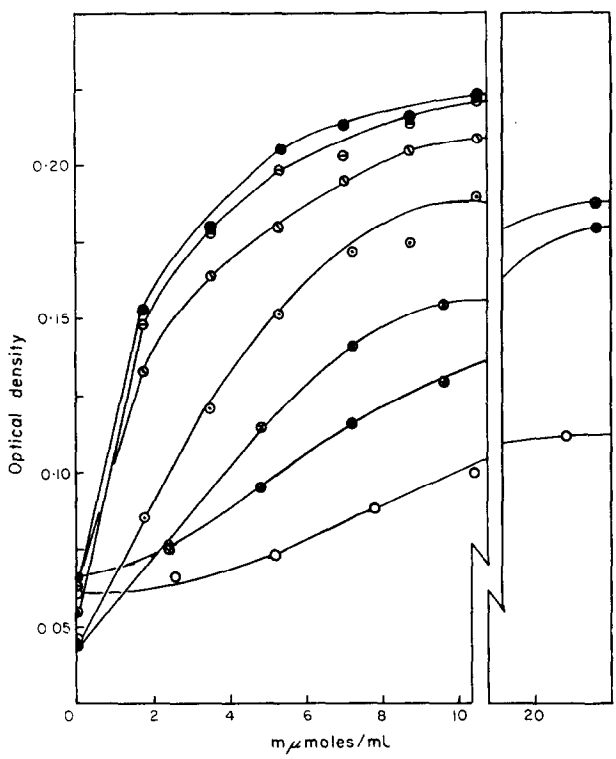

FIG. 1. Response of *Tetrahymena* to sterols and to sterol glycosides in the presence of 6-methylpurine: ○ cholesterol; ⊕ stigmasterol; ⊗ β-sitosterol; ○ stigmasterol glucoside; ⊘ cholesterol glucoside; ⊖ β-sitosterol glucoside; ● SII (calculated as β-sitosterol glucoside).

Although there are numerous reports of the isolation of sterol glycosides from various natural materials such as sweet potato,[7] soy beans,[8, 10] snakeroot,[3] cottonseed,[11] orange juice,[12] grapefruit pulp,[13] corn oil,[14] peanuts,[15] rapeseed and linseed,[16] only Ma and Schaffer[13] report biological activity in the inhibition of the growth of *Fusarium lini* by the glycoside. The present report appears to be the first to describe growth-promoting activity by the sterol glycosides which is greater than that of

the parent sterols. It is possible that the greater activity may be due to solubility differences or greater ease of permeation of the glycosides into living cells, as compared to that of the free sterol. This also appears to be the first time that the presence of sugars other than glucose in such sterol glycosides has been detected. It is apparent, however, that the exact nature of the glycone is of relatively small importance in determining the biological activity of sitosterol glycosides.

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