of the ridge (*9*) show several lineations in the form of strings of abyssal hills (ridges?) and regional offsets in bathymetry, which conform in trend and general position to an extension of the Murray Fracture Zone.

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Diosgenin and β-Sitosterol: Isolation from Solanum Xanthocarpum Tissue Cultures

Abstract. *Diosgenin and β-sitosterol were isolated from* Solanum xanthocarpum *callus, crystallized, and chemically characterized. That these metabolites, particularly diosgenin, form in significant amounts in tissue culture may prove useful.*

Formation, in tissue culture, of nicotine in *Nicotiana* (*1*), tropane alkaloids in *Datura* (*2*), substances similar to digitalis glycosides in *Digitalis* (*3*), vinca alkaloids in *Catharanthus* (*4*), reserpine in *Alstonia* (*5*), indole alkaloids in *Ipomoea* (*6*), visnagin in *Ammi* (*7*), formononetin in *Cicer* (*8*), and solasonine in *Solanum* (*9*) has been observed.

Steroids occur in solanaceous plants, and a few have been extracted from tissue cultures of *Nicotiana* (*10*). We now report on the isolation and identification of β-sitosterol and diosgenin from callus tissues of *Solanum xanthocarpum* (Solanaceae). Actively proliferating calli raised from the aseptic culture of young defoliated shoots on Murashige and Skoog's basal medium fortified with growth supplements (*11*) were used.

β-Sitosterol was isolated as follows. A portion (25 g) of oven-dried (60°C for 48 hours) callus was powdered and extracted in a Soxhlet apparatus with chloroform for 24 hours. The extract was concentrated to dryness and chromatographed on a column (1 by 10 cm) of silica gel (< 0.08 mm, E. Merck); the column was eluted with a benzene–ethyl acetate gradient. Fractions containing the steroid component were pooled and purified by thin-layer chromatography (silica gel G, E. Merck) with the following solvent systems: benzene and ethyl acetate (85:15); and chloroform and ethyl acetate (95:5). Fifteen milligrams of a homogeneous product was obtained and purified by crystallization from methanol (plates, 10 mg; m.p. 137°C). Elemental analysis showed 83.8 percent of carbon and 12.2 percent of hydrogen. The formula $C_{29}H_{50}$ requires 84.0 percent of carbon and 12.2 percent of hydrogen. The color reactions with $SbCl_3$ and concentrated H_2SO_4 indicated that the compound was a 3-β-hydroxy-Δ^5-steroid; this structure was confirmed by a positive Liebermann test.

The infrared (IR) spectrum of this product was superimposable with that of β-sitosterol. An acetyl derivative of the compound was prepared with acetic anhydride and pyridine. On crystallization from methanol, it melted at 126°C. No depression was observed in the melting point of the parent compound or its acetate on admixture respectively with authentic β-sitosterol or its acetate, thereby providing conclusive proof of the formation of β-sitosterol as a major product in tissue cultures of *S. xanthocarpum*.

Diosgenin was isolated as follows. The residue after the chloroform extraction was extracted with 95 percent ethyl alcohol in a Soxhlet apparatus. The solvent was removed, the residue was hydrolyzed (3 percent HCl for 3 hours), and the hydrolyzate was extracted with chloroform (4 by 25 ml) after neutralization with ammonia. The concentrated extract was chromatographed on silica gel column (< 0.08 mm, E. Merck) with chloroform and ethyl acetate mixtures. The fractions that gave positive $SbCl_3$ and concentrated H_2SO_4 color tests were combined and further purified by thin-layer chromatography on silica gel G with chloroform, and chloroform–ethyl acetate (95:5). The IR spectrum of the crystalline product (2 mg) was superimposable with that of authentic diosgenin, thus establishing the identity of the compound. On a thin-layer plate its behavior was like that of the reference sample. The acetyl derivative of the product showed the same R_F value as that of diosgenin acetate, further confirming the isolated compound as diosgenin.

Table 1. Steroid content from berries and tissue cultures of *Solanum xanthocarpum*.

Source	β-Sitosterol (%)	Diosgenin (%)
Berries	0.017	0.001
Tissue cultures	.04	.008

Chemical examination of *S. xanthocarpum* berries also resulted in the isolation of diosgenin, a compound already reported from the species (*12*). But the predominant constituent was β-sitosterol, known to occur in the related species *S. khasianum* (*13*). The steroidal content from tissue cultures as compared with the berries is given in Table 1.

Although only trace amounts of the steroidal alkaloid solasonine were detected earlier (*9*), the tissue cultures of *S. xanthocarpum* have yielded β-sitosterol and diosgenin in quantities much higher than that obtained from the growing plant.

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