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SHORT COMMUNICATION

PRESENCE OF DIOSGENIN AND ABSENCE OF SOLASODINE IN TISSUE CULTURES OF *SOLANUM LACINIATUM**

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Abstract—Diosgenin was isolated from *Solanum laciniatum* Ait callus cultures; its quantity was identical with that of field plants. However, the main steroid of the species, solasodine was not detectable in the callus.

OF THE characteristic plant steroids, the accumulation of the neutral sapogenin diosgenin in tissue cultures of *Dioscorea* species has been investigated in greatest detail.[1,2] In general, the formation and accumulation of both neutral and basic sapogenins are characteristic of *Solanum* species.[3] It was thus interesting to examine if this ability of *Solanum* species to accumulate both neutral and basic sapogenins under field conditions would persist under the conditions of tissue culture. Until the present report the only published data concerned one *Solanum* species, i e. *S. xanthocarpum* which in tissue culture formed both the neutral sapogenin diosgenin[4] and the basic sapogenin solasodine.[5]

Because of the significant solasodine content *Solanum laciniatum* Ait. (Solanaceae) provides the basic material of steroidal hormone production in countries of the temperate zone. The species contains 0 01–0·1 % of the dry wt as diosgenin in addition to 1 0–1·5 % of solasodine.[3,6,7] Analyses were carried out of callus cultures of *Solanum laciniatum*. The results obtained with tissues of various ages, between 4 to 12 weeks were practically identical in all 12 examinations performed.

The presence of sterols, neutral and basic sapogenins in callus cultures of the species was investigated in methanolic extracts after hydrolysis by TLC; the detected compounds were isolated by preparative TLC and the quantities were determined photometrically.

The isolated neutral sapogenin was identified as diosgenin by comparison on TLC of the compound and its acetate with authentic material (same R_f value in five solvent systems, same colour reactions) Quantitative examination showed that the callus contain diosgenin in about the same order as the original field plants (*ca.* 0 01 % on a dry wt. basis).

The presence of C_{25}-epimer of diosgenin and its saturated analogues was examined in two TLC systems. When the derivative obtained by acetylation was chromatographed in the solvent dichloromethane-toluene, a spot appeared that showed the same R_f and reactions as

* 'Examination of Steroids of Plant Origin' Part VI in the series For Part V see Ref 1.

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diosgenin acetate, in this solvent, yamogenin acetate showed a favourable separation. However, in our substance, no identical constituent was found. The saturated–unsaturated 'critical pairs' of steroids can be separated, often as acetates, by modified TLC methods, such silver-nitrate-treated plates,[8] impregnation with sodium acetate,[9] continuous development,[10] bromination.[11] It was found, that by simple TLC with the solvent benzene–ether (1·1) proved to be useful separation of the 'critical pairs' of free 3β-monohydroxy neutral sapogenins diosgenin–tigogenin and yamogenin–neotigogenin. The callus culture contained no constituent identical with tigogenin or neotigogenin, respectively.

No trace of solasodine was found in the tissues of various ages The sentivity of the method used was such that 1 μg/g of solasodine would have been detected had it been present in the callus, i e. one ten thousandth of the amount in field plants.

The presence of some bound sterols (about 0 01 %) was shown in addition to a significant amount (about 0·1 %) of free sterols. Therefore, according to the results presented, the neutral sapogenins are formed in tissue culture of *Solanum laciniatum*, but, formation and accumulation of basic sapogenins, which are by two to three orders greater in the field, do not occur.

EXPERIMENTAL

Tissue Culture

The tissue clone was isolated from a shoot raised under sterile conditions and the callus tissue arising from the cutting surface was taked on. Incubation was performed at 28° under the naturally changing light conditions for 4–12 weeks in surface culture on solid medium. Basal medium was Murashige and Skoog's[12] 'revised medium', without 'edamine' and with 0 5 ml/l A–Z microelement solution and with 1 mg/l gibberellic acid (GA_3) at a pH of 5 7 The daily growth rate was 180 mg/day, the starting weight amounted to 200 mg

Extraction

10 g of the dried (60°) and powdered callus was extracted extensively with hot aq. MeOH (70%). The combined extracts were shaken with light petroleum (b.p. 40–60°) and the petrol extract was examined for free sterols. The MeOH phase was evaporated, hydrolyzed by acid, purified in the manner described previously [1,3,13]

Chromatography

The samples for preparative TLC were applied to plates coated with 2 mm Silica gel G The plates were developed in the solvent cyclohexane–ethyl acetate–H_2O (60 40.0·1) and the separated sapogenin eluted with $CHCl_3$–MeOH (1·1). The solvents and reagents used for identification of diosgenin was described previously.[13]

TLC separation of C_{25}-epimers of sapogenin acetates (acetylation with acetic anhydride in pyridine): plates coated with 0·25 mm Silicagel G, solvent· dichloromethane–toluene (70.30, twice) Separation of saturated–unsaturated 'critical pairs' of sapogenins: plates coated with 0 25 mm Silica gel G, solvent: benzene–ether (50 50)

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