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《Original》

The Metabolism of (4-^{14}C) Cholesterol on Photoperiodism in Solanum andigena

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Abstract

(4-^{14}C) Cholesterol was administered to the leaves of *Solanum andigena* during photoperiodic induction. Radioactive products converted from cholesterol were studied by thin-layer chromatography and gas-chromatography. The major products from cholesterol were shown to be esterified cholesterol in lipid and solanine in the aquoeus ethanolic phase in SD-and LD-leaves. The radioactive solanidine was isolated by chromatography and crystallized to constant specific activity. Short-day condition did not stimulate the conversion of cholesterol into solanine in the leaves, but both groups of leaves converted cholesterol into solanine at the about same rate. Incorporation of radioactivity into aqueous ethanolic phase of tubers and stolons, containing steriod glycoalkaloid, was very much higher than that into lipid phase, contrary to those in the leaves and the stems.

요 약

*Solanum andigena*의 광주성 주기중의 방사성 코레스테로올의 대사를 연구하였다. 대사산물로서 방사성 솔라닌과 그 가수분해물 솔라니딘을 분리 결정화하였으며, 코레스테로올은 이 식물체 내에서 형성되는 애스테르화된 형태로 분리하였다. 단일 및 장일식물간의 코레스테로올 대사에는 뚜렷한 차이는 찾아 볼 수 없으나. 괴경(塊莖)중의 스테로이드 알카로이드에 코레스테로올이 관여하고 있는 것으로 논의되었다.

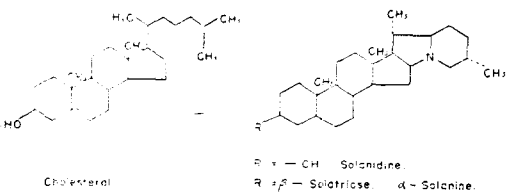
Introduction

Solanum andigena is a wild species of potato in which the tuberization response is strongly photoperiodic; plants grown under short-day condition will tuberize. It is presumed that a tuberization hormone is produced in leaves, the locus of the photoperiodic reaction.

We have recently studied the changes which occur in the levels of free sterols, their esters and glycosides in the leaves of *Solanum andigena* when the plants are transferred from long-day (LD; 16hr of light, 8 hr of darkness) to short-day (SD; 8 hr of light, 16 hr of darkness).[1] This transfer caused an initial sharp decrease in the levels of β-sitosterol and cycloartenol and a rise in the level of cholesterol, however, after about three weeks of short-day condition the

levels of β-sitosterol and cycloartenol returned to those of the LD leaves, and the levels of cholesterol decreased. It was also demonstrated that (2-^{14}C) mevalonic acid was efficiently incorporated into cholesterol in the leaves of the plant.[2]

Since it is known that SD conditions stimulate the synthesis of the glycoalkaloid, solanine, in *Solanum* tubers[3] and that (4-^{14}C) cholesterol is converted into solanidine, the aglycone of solanine, in *Solanum tuberosum*[4], it was thought that the marked change in the level of cholesterol in the leaves of *Solanum andigena* could be related to its conversion to steriod alkaloid such as solanine.



Cholesterol

R = — CH Solanidine
R = β — Solatriose α — Solanine

Experimental

Plant Material: Eight plants of *Solanum andigena* were grown from cuttings taken from LD-plants for 4 weeks under LD condition, and then divided into two groups of 4 plants. One group was transferred to SD conditions and (4-^{14}C)-cholesterol administered to its leaves over a period of 3 weeks; the leaves were harvested 1 week later. The other group remained under LD condition. (4-^{14}C)-cholesterol was administered to its leaves over a period of 3 weeks and the leaves were also harvested 1 week later.

Administration of cholesterol: The method of administration of the radioactive cholesterol was based on that of Bennett and Heftmann[5]. The leaves were first washed with a 0.1% (v/v) aqueous solution of Tween 80, then with distilled water and finally allowed to dry. Both surfaces of the leaves were then painted with 4.167 μCi of (4-^{14}C) cholesterol (153 μCi/mg, The Radio chemical Centre, Amersham, England) dissolved in 1 ml of 60% (v/v) aqueous ethanol containing 0.04% (v/v) Tween 80. Previous tests had shown that this aqueous ethanol solution caused no visible harm to the leaves. This treatment was repeated at intervals of, alternatively, 2 and 3 days, a further 5 times; 25 μCi of (4-^{14}C) cholesterol had been administered to the leaves of each group of plants. All compound leaves, except the first, were treated in this way. After the final application of radiocative cholesterol the leaves were sprayed with a 50% (v/v) solution of silicone oil (MS 200/100 cs) in light petroleum (b. p. 40-60°C) to promote absorption of the cholesterol and to reduce its rate of oxidation on the leaf surface.

Isolation and Purification of Solanine: Four weeks after the first application of the (4-^{14}C) cholesterol the leaves from the two groups of plants were harvested. They were washed with light petroleum (b. p. 40-60°C) and ethanol to remove residual, surface cholesterol. The two batches of washed leaves (SD, 72g wet wt.; LD, 78g wet wt.) were homogenized in 200ml of boiling 95% ethanol and then filtered. The residue was rehomogenized with 100ml of 60% (v/v) aqueous ethanol containing 5% (v/v) acetic acid and allowed to stand overnight at 4°C, It was then filtered and the filtrate combined with the 95% ethanol extract. The combined ethanolic extracts were diluted with 200ml of water and extracted twice with 500ml of light petroleum. (Lipid phase) The residual aqueous ethanol phase was then concentrated to 40/ml under reduced pressure and the solanine siolated from it by the method of Lampitt et al[6]. Four grams of sodium sulphate were added and the extract was warmed on a water bath for 30 min. It was then cooled and 1 ml of 20% (v/v) sulphuric acid added. The mixture

was centrifuged and the supernatant solution decanted off, made alkaline (pH 10.2) with strong ammonia and allowed to stand overnight at 4°C. The resulting precipitate of crude solanine was isloated by centrifugation, washed with 2% (v/v) ammonia. The crude solanine was redissolved in 5% (v/v) acetic acid and purified by reprecipitation with ammonia three times.

Preparation of Solanidine: The solanine was then hydrolyzed by refluxing for 5 hr in 10ml of a mixture of ethanol, water and sulphuric acid (48.6 : 48.6 : 2.8 v/v/v)[7]. The hydrolysate was cooled, neutralized with ammonia and the solanidine extracted with chloroform. The solanidine was then purified by chromatography on thin layers of kieselgel G using a mixture of chloroform, methanol and glacial acetic acid (85 : 13 : 12 v/v/v) for development; markers of authentic solanidine (Fluka AG, chemische Fabrik, Buchs, Schweiz.) were run on each plate. After development solanidine were detected with saturated antimonytricholride in chloroform. The solanidine eluted from thin layers was crystallized from ethanol.

Radioassay: Radioactive samples were counted in a Packard TriCarb Liquid Scintillation Spectrometer Series 314E and thin layers scanned with a Desaga-Radioactive chromatogram scanner 12-2 as previously described[2].

Results and Discussion

Two groups of cuttings taken from *Solanum andigena* were grown for 4 weeks under LD condition. Both groups were treated with (4-^{14}C) cholesterol during photoperiodic cycles. Considerable amounts of radioactive cholesterol were absorbed through the surfaces when applied to the leaf by the method as described in the Experimental.

The lipid phase extracted with light petroleum contained approximately 72.3% of radioactivity in the LD-leaves, 70% in the SD-leaves, absorbed. The lipid phases of the leaves were concentrated and chromatographed on columns of Brockmann grade III neutral alumina, developing by step-wise elution with 2% (v/v) diethyl ether in light petroleum (b. p. 40-60°C); (E/P), 20% E/P and 95% ethanol. Column fractions (3×10^6 counts/min in LD, 5.5×10^5 counts/min in SD-leaves) eluted with 2% E/P were chromatographed on thin-layer of Kieselgel G impregnated with Rhodamin 6G , developed with petroluem ether (b. p. 40-60 C). Thin-layers were then scanned for ^{14}C radioactivity. Highly radioactive Zone was at the Rf value of 0.07, identical with that of sterol ester.

Table 1. Incorporation of radiactivity into various portions of *Solanum andigena* when 25 μCi of (4-^{14}C) cholesterol was applied to the leaves of each group.

	Radioactivity incorporated (counts/min.)	
	Aqueous ethanol phase	Lipid phase
LD-leaves	3, 350, 900	8, 742, 000
LD-stems	120, 200	85, 700
LD-stolons	12, 100	1, 500
SD-leaves	3, 378, 000	7, 873, 000
SD-stems	34, 200	64, 000
SD-stolons & tubers	11, 200	6, 600

The main radioactive matter of the thin-layer was eluted out, hydrolysed with ethanolic solution containing KOH as previously described[2], extracted three times with diethyl ether and washed alkali-free. The ether extract was dried over anhydrous Na_2SO_4 and evaporated. The extract was chromatographed on thin-layer of Kieselgel G impregnated with Rhodamin 6G, developed with cholroform. The thin-layer was scanned for ^{14}C radioactivity. The main radioactive zone was identical with the Rf value of 4-demethyl sterols. The analysis of the matter by the preparative gas-chromato-

graphy[2] was shown to be cholesterol. The fractions eluted from alumina column with 20% E/P contained 1.4×10^5 counts/min in LD-leaves and 1.9×10^6 counts/min in SD-leaves of radioactivity incorporated. They were chromatographed on thin-layer with chloroform. Thin-layer radioscannings showed that these were three radioactive zones at RF values of 0.35 (zone 1), 0.82 (zone 2) and 0.95 (zone 3) in both groups. Zone 1 was identical with cholesterol in mobility and in the analysis by the preparative gas chromatography. Zone 2 was coincident with cholesterone in mobility on thin-layers. Johnson et al[10]. demonstrated that one of the major transformation products of cholesterol in *Solanum tuberosum* is cholest-4-one-3-one. Zone 3, which could be eluted with 6% E/P from the alumina column, was not investigated in this experiment.

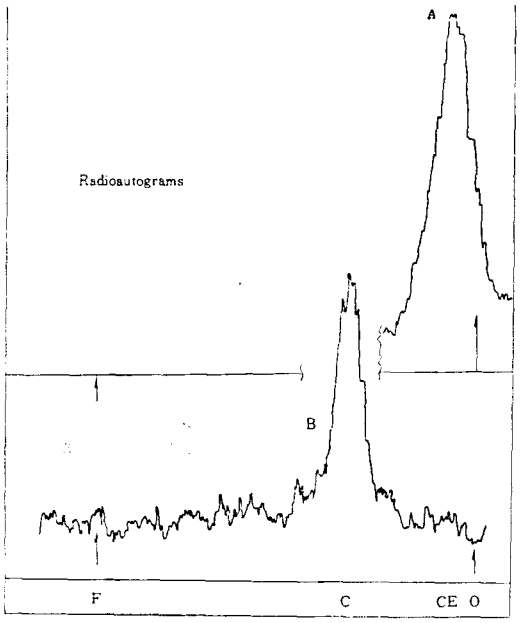


Fig. 1. Radioautograms of 2% E/P column fraction from *Solanum andigena*.
A: A kieselgel G plate impregnated with Rhodamin 6G was developed with light petroleum (b. p. 40–60 C)
B: developed with chloroform after alkaline hydrolysis
O, Origin; F, Solvent Front; CE, Esterified cholesterol; C, Cholesterol

Solanidine was prepared from solanine by acid hydrolysis. The hydrolysate was made slightly alkaline with ammonia. The solanidine was extracted with chloroform and then purified by chromatography on thin-layers of kieselgel G. Thin-layer radioscannings of the TLC plates showed that there were four radioactive zones in the solanine hydrolysate; One of them (Rf.; 0.2) was found to be the intact solanine. During acid hydrolysis of solanine, some of solanidine is converted to $\Delta^{3,5}$ -solanidine[10], which reacts red with $SbCl_3$ without heat and can be found on thin-layers along with solanidine. Authentic solanidine was run on each plates as markers and detected with $SbCl_3$ after heating. The radioactive zone corresponding to solanidine was isolated from thin-layers. This procedure gave a yield of 3.7 mg and 4.7 mg of solanidine (m.p. 219°C) from SD-and LD-leaves respectively. Both samples of the compound were diluted by the addition of 10 mg of non-radioactive, authentic solanidine, and crystallized from ethanol to constant specific radioactivity.

The solanidine from the SD-leaves after dilution with non-radioactive solanidine had a specific radioactivity of 10.6×10^4 counts/min/m mole whilst that from the LD-leaves had specific radioactivity of 14.4×10^4 counts/min/m mole; these values became 39.06×10^4 and 45.07×10^4 counts/min/m mole respectively after correction for dilution.

This result indicates that short-day conditions do not stimulate the conversion of cholesterol into solanidine in the leaves of *Solanum andigena:* nor do they depress it. Table 1 shows that incorporation of the radioactivity into aqueous ethanol phase in SD-plants is approximately twice of that of the lipid phase. This result suggests that the biosynthesis of steroid alkaloids in tuber may be much higher than

that of sterol ester.

There are differences in the rate of alkaloid biosynthesis in various tissue . McKee reported that solanine was produced in wounded potato.[11] The steroid-glycoalkaloids, α-solanine and α-chaconine, were the major fungitoxic compounds in the extracts of potato peel.

In this standpoint, α-solanine biosynthesis in *Solanum* tuber remain to be investigated.

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