

but this is unlikely in view of the above experimental results—especially because this band can be reversibly separated from the pigmented calcium stone without associating the bilirubin skeleton. The 1702 cm^{-1} band can thus not be utilized for the analyses of gallstones.

The detailed results together with a discussion will be published in the Tohoku Journal of Experimental Medicine.

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Department of Surgery,
Tohoku University School of Medicine,
Kitayobancho, Sendai

Noriyoshi Suzuki (鈴木範美)
Masayuki Toyoda (豊田政幸)

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Regulation of Nicotine Synthesis in Tobacco Callus Tissue*¹

In a previous communication,¹⁾ it was reported that nicotine and anatabine are found in tobacco callus tissue grown on a medium containing 3-indoleacetic acid (IAA). The present communication deals with the presence of phytosterols such as β -sitosterol, stigmasterol, and campesterol, and the absence of nicotine and anatabine, in tobacco callus tissue grown on an agar medium containing 2,4-dichlorophenoxyacetic acid (2,4-D) instead of IAA, and the effect of IAA and 2,4-D on the formation of nicotine and phytosterols.

The callus derived from stems of *Nicotiana tabacum* var. Bright Yellow was subcultured and maintained on agar medium containing White's medium, 0.5% yeast extract, and 1 p.p.m. of 2,4-D. The callus was cultured by the same condition as reported previously.¹⁾ Thirteen hundred grams of fresh callus collected was homogenized in a Waring blender with 1.3 L. of cold acetone and allowed to stand for 7 days. Solid material in the homogenate was removed by filtration and the filtrate, after acidification, was carefully concentrated to a small volume under reduced pressure. The concentrated solution was extracted three times with methylene chloride. The methylene chloride solution was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was designated as Fraction A. The aqueous solution after extraction with methylene chloride was made alkaline with 6N NaOH and reextracted with methylene chloride. The methylene chloride solution was dried over anhydrous sodium sulfate and evaporated gently to dryness. The residue was designated as Fraction B. Further, the aqueous solution after extraction with methylene chloride, after acidification, was extracted with water-saturated butanol. The butanol solution was evaporated to dryness under reduced pressure. The brownish residue thereby obtained was designated as Fraction C. Fraction A, B, and C were each dissolved in a small amount of acetone and submitted to thin-layer chromatography and gas-liquid chromatography.

*¹ This work was presented at the Eleventh Pacific Science Congress, Tokyo, August, 1966.

1) T. Furuya, H. Kojima, K. Syono: This Bulletin, 14, 1189 (1966).

Thin-layer chromatogram of Fraction A on Silica Gel G plate developed with benzene-ether (8:2) gave many spots, one (R_f 0.33) of which corresponded in mobility to the mixture of β -sitosterol, stigmasterol, and campesterol as illustrated in Fig. 1.

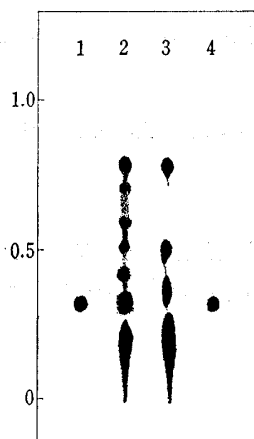


Fig. 1. Thin-layer Chromatogram of Phytosterols from Tobacco Callus

1. Campesterol and β -sitosterol
2. 2,4-D callus
3. IAA callus
4. Stigmasterol

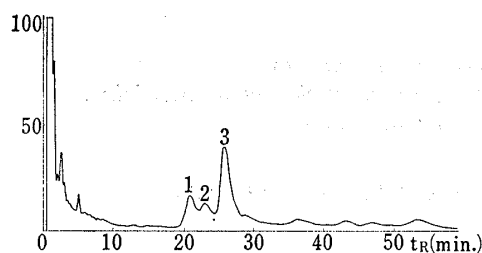


Fig. 2. Gas-liquid Chromatogram of Phytosterols from Tobacco Callus

1. Campesterol
2. Stigmasterol
3. β -Sitosterol

By gas-liquid chromatography,²⁾ three main peaks in Fraction A were also identified with the authentic samples of β -sitosterol (t_R 26.1 min.), stigmasterol (t_R 22.6 min.), and campesterol (t_R 20.8 min.). The gas-liquid chromatogram is shown in Fig. 2 and the operating conditions were as follows: A Shimadzu Model GC-IC gas chromatograph equipped with a hydrogen flame ionization detector and glass column (4 mm. \times 1.875 m.) packed with 1% SE-30 on Gas-Chrom Q (80~100 mesh) were used. Nitrogen flow rate was 84.0 ml./min., column temperature 240°, flash heater temperature 300°, and detector temperature 250°.

Thus, the presence of β -sitosterol, stigmasterol, and campesterol in Fraction A was clearly demonstrated by thin-layer and gas-liquid chromatography, and we were not able to confirm the presence of nicotine and anatabine in Fraction B by the previous method.¹⁾

Ourisson and his co-workers³⁾ recently reported the absence of nicotine and the presence of phytosterols and related compounds in habituated tobacco callus tissue. Their result is similar to those mentioned above.

On the other hand, in the callus grown on a medium containing IAA, a large amount of nicotine and a small amount of anatabine were already found from Fraction B by us, and we were not able to identify β -sitosterol, stigmasterol, and campesterol from Fraction A by the same method as described above.

Scopoletin⁴⁾ and its glucoside, scopolin⁴⁾, were respectively identified by thin-layer chromatography from Fraction A and C in the callus grown on the two kinds of medium used.

The chemical constituents obtained so far from tobacco callus are briefly summarized in Table I.

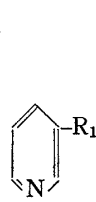
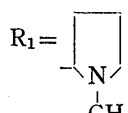

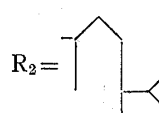
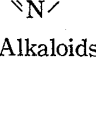
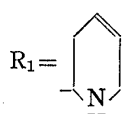
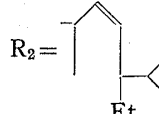
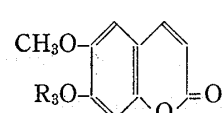
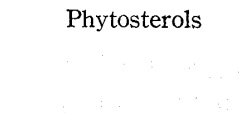
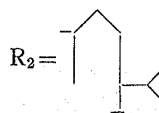
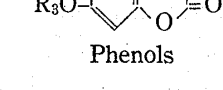
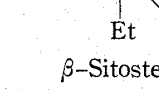
It is very interesting to note that syntheses of alkaloids such as nicotine and anatabine, and of phytosterols such as β -sitosterol, stigmasterol, and campesterol, in

2) T. Murakami, H. Itokawa, A. Matsushima, N. Ikekawa: *Yakugaku Zasshi*, **83**, 427 (1963).

3) P. Benveniste, L. Hirth, G. Ourisson: *Phytochemistry*, **5**, 31 (1966).

4) J.A. Sargent, F. Skoog: *Physiol. Plantarum*, **14**, 504 (1961).

TABLE I. Constituents in Tobacco Callus

Compound		Callus	IAA	2,4-D	Compound		Callus	IAA	2,4-D
Alkaloids		R ₁ = 	+	-	Phytosterols		R ₂ = 	-	+
		Nicotine					Stigmasterol		
		R ₁ = 	+	-				-	+
	Anatabine					Et			
Phenols		R ₃ = H	+	+			R ₂ = 	-	+
		Scopoletin					Et		
		R ₃ = Glucose	+	+			-	+	
	Scopolin					Et			

tobacco callus have been shown to be markedly influenced by the supply of IAA and 2,4-D in the nutrient medium.

More detailed chemical and biosynthetic investigations are now in progress to determine the possible causal relationship between the action of IAA and that of 2,4-D in these phenomena.

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College of Pharmaceutical Sciences,
Kitasato University,
Shiba, Minato-ku, Tokyo

Tsutomu Furuya (古谷 力)
Hisashi Kojima (児嶋 脩)
Kunihiko Syono (庄野邦彦)

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New Constituents of *Chamaecyparis formosensis* MATSUM.¹⁾

During the reinvestigation of the terpenoid constituents of the Benihi tree (*Chamaecyparis formosensis* MATSUM., Cupressaceae, grown in Taiwan), a novel nor-sesquiterpenoid, chamaecynone and related compounds were isolated.^{1,2)} Chamaecynone and freelinene reported by Massy-Westropp, *et al.*³⁾ are the first examples of acetylenic

1) Presented at the general local meeting of the Hokkaido district of the Chemical Society of Japan, at Sapporo, in July, 1965; abstract paper, pp. 11~12.

2) T. Nozoe, Y. S. Cheng, T. Toda: Tetrahedron Letters, 1966, 3663.

3) R. A. Massy-Westropp, G. D. Reynolds, T. M. Spotswood: Tetrahedron Letters, 1966, 1939.