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Microbial Conversion of Tall Oil Sterols to C_{19} Steroids

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Mycobacterium sp. NRRL B-3683 converted tall oil sterols to C_{19} steroids as efficiently as it converted soybean sterols.

In recent years, the demand for steroid drugs has increased. The increase will likely continue because of expanding demands for contraceptives, corticosteroids, and geriatric drugs. The primary raw materials for producing steroid drugs are diosgenin from *Dioscorea* spp., stigmasterol from soybeans, and cholesterol from animal sources. The increased demands for steroid drugs are complicated by a shortage of diosgenin. Plant sterols (phytosterols), such as sitosterol and campesterol, are potential starting materials. They are not now used, however, because of difficulty in removing the aliphatic side chain.

Tall oil, a by-product of the kraft (sulfate) pulping of pinewood chips, contains about 3% steroid compounds. These steroids consist of about 17 different compounds, of which sitosterol and campesterol comprise about 85% (2). In 1974, the United States produced about 800,000 tons of tall oil. Thus, approximately 20,000 tons of tall oil phytosterols was potentially available as raw material for steroid drug production. These are concentrated in the pitch that remains after crude tall oil is distilled. Procedures for obtaining sterols from this tall oil pitch have been developed (D. V. Julian, U.S. Patent 3,840,570, 8 October 1974). Pitch is now used mainly for its fuel value; in contrast, soybean foots, the source of soybean sterols, is more expensive than pitch because of its added value as an animal feed.

Microbial removal of the aliphatic side chain of phytosterols has been studied for some time (3) and offers a promising method for use of these sterols. Recently, Marsheck et al. (4) reported that *Mycobacterium* spp. NRRL B-3683 and NRRL B-3805 converted cholesterol, sitosterol, and stigmasterol to androsta-1,4-diene-3,17-dione and androst-4-ene-3,17-dione without the use of the selective metabolic poisons needed when other microorganisms were used for this transformation (5). Indeed, sitosterol is fermented to androst-4-ene-3,17-dione by this method for use as an intermediate in producing the steroid drug spironolactone (1). In this work, we have extended the study of Marsheck et al. to show that tall oil sterols are also converted by B-3683 to C_{19} steroids. Thus, tall oil does not contain components that hinder its fermentation by this organism.

A sample of sterols, crystallized from tall oil and used in the experiments reported here, was shown by a combination of gas-liquid chromatography (GLC) and nuclear magnetic spectroscopy to contain compestanol (1%), campesterol (8%), stigmastanol (11%), sitosterol (72%), stigmasterol (1%), wax alcohols (5%), and triterpenes (cycloartenol and 24-methylenecycloartanol) (2%). The sterol sample was dissolved in *N,N*-dimethylformamide (100 g/liter). Portions were added to 24-h cultures of *Mycobacterium* sp. NRRL B-3683 growing in nutrient broth (Difco) to give final concentrations of 1 g/liter. The broth was supplemented with 1 g of yeast extract (Amber Laboratories, Inc.) per liter and 1 g of inositol (Sigma Chemical Co.) per liter. The fermentations were carried out in cotton-plugged, 2-liter Erlenmeyer flasks containing 200 ml of medium, which were placed on a rotary shaker (300 rpm) at 30°C. After 2, 4, 6, and 8 days of incubation, the content of one of the replicate flasks was extracted twice with 75 ml of $CHCl_3$. The extracts were concentrated to dryness, trimethylsilylated with *N,O-bis*-(trimethylsilyl)-acetamide, and analyzed by GLC on a glass column (1.8 m by 4 mm [inner diameter]) packed with a mixture of 2.5% SE-30 and 1.5% QF-1 on 100/120-mesh Gas Chrom Q. *n*-Hexacosane was used as an internal standard. Relative response factors for the various components were used for calculations based on the GLC analyses. By these GLC methods, campesterol and campestanol are eluted as a single peak, and sitosterol and stigmastanol are eluted as a single peak.

Fermentations with crude sitosterol from soybeans (Sigma Chemical Co.) were conducted similarly. GLC analysis of this material showed the following: campesterol/campestanol (35%), stigmasterol (4%), and sitosterol/stigmastanol (61%).

TABLE 1. *Conversion of tall oil phytosterols by Mycobacterium sp. NRRL B-3683*

Sterol source	Incubation period (days)	Percent conversion to:[a]			
		ADD	AED	PEO	PDO
Soybean	2	29	1	t	3
	6	54	1		2
	8	38	1		1
Tall oil[b]	2	28	1		2
	4	46	3		3
	6	52	1		4
	8	48	1	t	3

[a] Percent conversion = (weight of product/weight of sterol added) × (molecular weight sitosterol/molecular weight product). ADD, Androsta-1,4-diene-3,17-dione; AED, androst-4-ene-3,17-dione; PEO, 20α-hydroxymethylpregn-4-en-3-one; PDO, 20α-hydroxymethylpregna-1,4-dien-3-one. t, Trace.

[b] Calculations are based on sample added minus wax alcohol and triterpene contents.

As the fermentations progressed, the tall oil and the soybean sterols were converted to androsta-1,4-diene-3,17-dione and to minor amounts of androst-4-ene-3,17-dione, 20α-hydroxymethylpregn-4-en-3-one, and 20α-hydroxymethylpregna-1,4-dien-3-one (Table 1). The pH of the fermentations was more than 8.5 after 6 days, which may account for the drop in conversions at the 8-day sampling. The wax alcohols in the tall oil starting material were metabolized within 2 days. Campesterol, stigmasterol, and sitosterol were utilized equally. However, Marsheck et al. (4) reported that γ-sitosterol, a campesterol-sitosterol mixture with campesterol predominating, was not utilized by this organism. Marsheck agrees that this observation must be in error (private communication) because β-sitosterol, a sitosterol-campesterol mixture with sitosterol predominating, is utilized.

Tall oil sterols are potentially available in large quantities at relatively minor cost and now are not utilized. They are converted to C_{19} steroids as efficiently as soybean sitosterol; thus they are a potential supplemental raw material for producing steroid-type drugs.

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