

Stereochemistry of Hydrogen Migration from C-24 to C-25 during Isofucosterol Biosynthesis in *Pinus pinea*

By FRANCESCO NICOTRA, FIAMMA RONCHETTI, and GIOVANNI RUSSO*

(Istituto di Chimica Organica dell'Università di Milano, Centro di Studio per le Sostanze Organiche Naturali del C.N.R.,
Via Saldini 50, 20133 Milano, Italy)

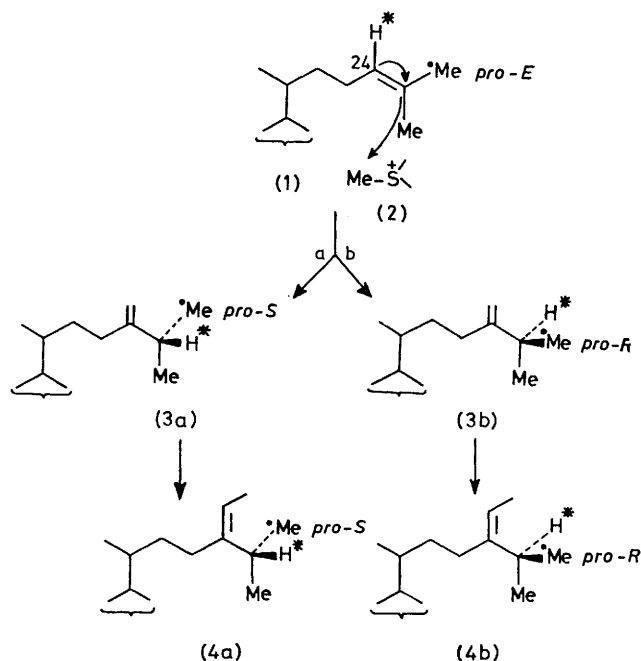
and GIUSEPPE LUGARO and MARILENA CASELLATO

(Laboratorio di Chimica degli Ormoni del C.N.R., Via M. Bianco 9, Milano, Italy)

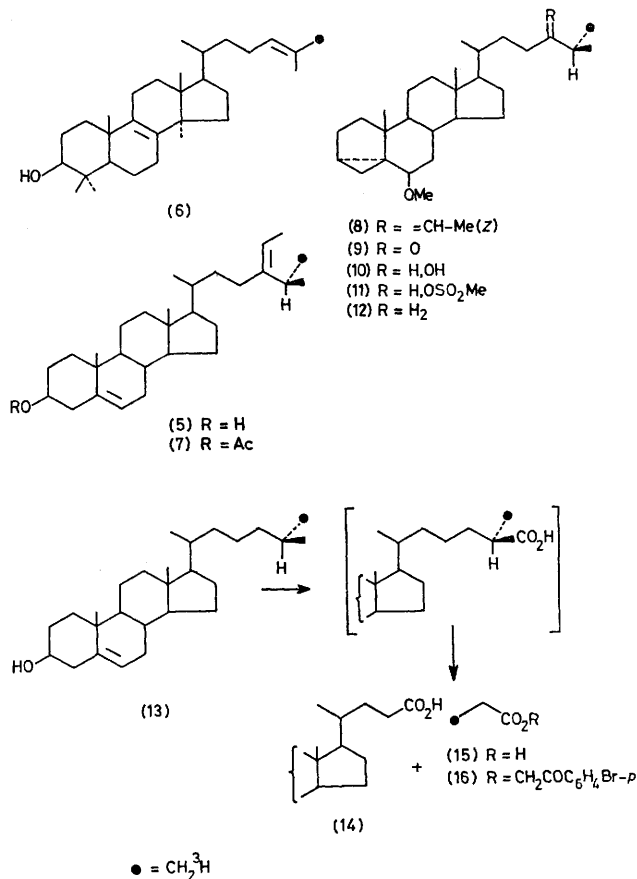
Summary During the biosynthesis of isofucosterol from lanosterol in *Pinus pinea* the stereochemistry of the hydrogen atom migration from C-24 to C-25 has been established.

THE C-24 ethylidene group of isofucosterol (5), a typical phytosterol of higher plants, arises by transmethylation of a $\Delta^{24(25)}$ precursor (1) which is converted, through the intermediacy of a 24-methylene-compound (3), into the 24-ethylidene sterol (4).¹ A key step of this process is the migration to C-25 of the hydrogen atom originally at C-24.²

From the stereochemical point of view, this migration can occur in two ways which lead to opposite configurations at C-25. In case (a) the *pro-E* methyl group of (1) becomes the isopropyl *pro-S* methyl group in the phytosterol side chain, whereas in case (b) the same methyl group assumes the *pro-R* position. Consequently, the stereochemistry of the migration of the hydrogen atom from C-24 to C-25 can be determined by examining the stereochemical fate of the above *pro-E* methyl group when the $\Delta^{24(25)}$ -intermediate (1) is transformed into isofucosterol (5). Moreover, if the stereochemical relationship between the methylation by S-adenosylmethionine and this hydrogen migration were discovered, the stereochemistry of the transmethylation process could be inferred.



As lanosta-8,24-dien-3 β -ol is known to be transformed into isofucosterol in *Pinus pinea*, we synthesized³ [26-³H]-lanosta-8,24-dien-3 β -ol (6) and administered it (1.64×10^9 d.p.m. of ³H) to 60 shelled seeds of *Pinus pinea*. The germination was allowed to proceed until the roots were 0.5–1 cm long, after which the germinated seeds were extracted and the sterol fraction isolated.¹ From this fraction pure isofucosteryl acetate (7) (2.30×10^6 d.p.m. of ³H) was isolated by acetylation and argentation chromatography. This compound was transformed into 6 β -methoxy-3 α ,5-cyclostigmast-Z-24(28)-ene (8) by hydrolysis,



tosylation, and treatment with MeOH-pyridine. Ozonolysis of (8) to the ketone (9), followed by reduction with NaBH₄, yielded the 24-alcohol (10), which was transformed into [26-³H]-6 β -methoxy-3 α ,5-cyclocholestane (12) by mesylation and reduction of the mesylate (11) with LiAlH₄. Purification of (12) by AgNO₃-silica gel preparative t.l.c. followed by hydrolysis with toluene-*p*-sulphonic acid afforded pure [26-³H]cholesterol (13) (2.76×10^5 d.p.m. of ³H), which was mixed with [26-¹⁴C]cholesterol (8.62×10^4 d.p.m. of ¹⁴C; ³H/¹⁴C ratio = 3.20:1). The doubly labelled sample was incubated with 6 g of rat liver mitochondria, which are known⁴ to transform cholesterol into bile acids (14) and propionic acid (15) by cleavage between C-24 and C-25 and stereospecific oxidation of the isopropyl *pro-S* methyl group, whereas the *pro-R* methyl group remains unaltered. The radioactive propionic acid was isolated from the incubation mixture by steam distillation and purified as its *p*-bromophenacyl derivative (16), which was crystallized from hexane and the label was counted (see Table). The data of the Table show the retention of

TABLE

Propionic acid (as *p*-bromophenacyl derivative) formed during the incubation with rat liver mitochondria of [26-³H;26-¹⁴C]-cholesterol (8.62×10^4 d.p.m. of ¹⁴C; ³H/¹⁴C ratio = 3.20)

	d.p.m. of ¹⁴ C/mmol	³ H/ ¹⁴ C ratio
3rd crystallization	7.94×10^3	2.57
4th crystallization	8.08×10^3	2.62
5th crystallization	7.89×10^3	2.60

81% of the tritium present in the isopropyl methyl groups of cholesterol, indicating that the label was mainly located on the *pro-R* methyl group of cholesterol (13), and hence of isofucosterol (5).

These data are consistent with the existence of a main biosynthetic pathway leading from lanosterol to isofucosterol in *Pinus pinea*, in which the migration of the hydrogen atom from C-24 to C-25 occurs *via* pathway (b) from the Δ^{24} precursor (1).

(Received, 29th July 1977; Com. 784.)

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