Synthesis of Episterol, 5-Dehydroepisterol and Their Deuterio-labeled Analogs

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Abstract : To identify and conduct metabolic studies on sterols in *Arabidopsis* dwarf mutants, episterol, 5-dehydroepisterol, [26,27-²H₆] 5-dehydroepisterol and [26,27-²H₆] episterol were synthesized from 3 β -acetoxycholest-5-en-24-one or its deuterio-labeled analog by introduction of the 5,7-diene group, olefination of the 24-oxo group with Tebbe reagent and reduction of 5,7-diene with sodium as key reactions.

Key words : synthesis, 5-dehydroepisterol, episterol, [26,27-²H₆] 5-dehydroepisterol, [26,27-²H₆] episterol

1 Introduction

The authors recently established an outline for the biosynthesis of phytohormones or brassinosteroids from a common phytosterol, campesterol 1¹⁾. During a study on dwarf mutants of *Arabidopsis thaliana* L., whose phenotypes were rescued by exogenous application of brassinosteroids, two novel dwarf mutants, *dwf 5* and *dwf 7*, were found to be defective in brassinosteroid biosynthesis and both of them were blocked before 24-methylenecholesterol 2 in sterol biosynthesis, based on the results of a feeding experiment with biosynthetic intermediates and particularly the quantitative analysis of endogenous sterols and brassinosteroids²⁾. To clarify blocked steps in the biosynthetic pathway, attention was initially directed to 5-dehydroepisterol 3 and episterol 4 (Fig. 1), situated just before 24-methylenecholesterol 2 in the sterol biosynthetic pathway^{3,4)}.

5-Dehydroepisterol 3 and episterol 4 were previously isolated from yeast⁵⁾ and *Mucor rouxii*⁶⁾ and this work proved to be a tedious and time-consuming. Sterols 3 and 4 are not commonly found in plants⁷⁾ and, thus, are not readily available from natural sources. The synthesis of 5-dehydroepisterol 3 and episterol 4 was previously

Fig.1 Structures of Campesterol and Related C₂₈ Sterols.

reported by Fryberg *et al.*⁸⁾ and requires long reaction steps for both sterols. The authors thus endeavored to develop a short-step synthesis of 5-dehydroepisterol 3 and episterol 4 for the identification and the metabolic study of the deuterio-labeled analogs.

2 Experimental

2-1 Analytical Methods

Melting points were determined under a hot-stage microscope (Yanagimoto micro melting point apparatus) and not corrected. ¹H-NMR spectra were recorded on a JEOL JMN-A 400 (400 MHz) or JEOL JMN-ECP 500 (500 MHz)

spectrometer in a deuterio-chloroform (CDCl_3) solution using tetramethylsilane as the internal standard. EI-MS and HR-EI-MS were measured with a JEOL SX-102 mass spectrometer at 70 eV. GC-MS was conducted with a Shimadzu GC-MS QP-5000 spectrometer equipped with a ULBON HR-1 fused silica capillary column, as described previously⁹.

2·2 Synthesis

2·2·1 Preparation of 3 β -hydroxycholesta-5,7-dien-24-one 7.

A solution of a known 3 β -acetoxycholest-5-en-24-one 5¹⁰ (167 mg, 0.378 mmol) in carbon tetrachloride (9 mL) was treated with *N*-bromosuccinimide (79 mg, 0.444 mmol) at 100°C under argon for 20 min. The reaction mixture was cooled to 0°C; the precipitate was filtered off and filtrate concentrated below 30°C to give a mixture of corresponding 7-bromides 6. The bromide in tetrahydrofuran (THF) (12 mL) was treated with tetra-*n*-butylammonium bromide (15 mg) at room temperature in the dark under argon for 1 h. A solution of tetra-*n*-butylammonium fluoride in THF (1 M, 1.5 mL) was then added to the system followed by additional stirring in the dark at room temperature under argon for 30 min. The work-up (ethyl acetate, EtOAc) gave a crude 3 β -acetoxycholesta-5,7-dien-24-one, which in THF (9 mL)

was treated with 5% KOH/methanol (MeOH) (3 mL) at room temperature in the dark under argon for 2 h. The work-up (EtOAc) gave a crude product, which was then applied onto eight plates of silica gel (20 cm x 20 cm, 0.5 mm thickness, Kieselgel 60F₂₅₄, Merck). The plates were developed with benzene-EtOAc (10 : 1, vol/vol). The silica gel band with R_F 0.31~0.36 was scraped off, eluted with EtOAc, filtered and concentrated below 30°C to afford 3 β -hydroxycholesta-5,7-dien-24-one 7 (71 mg, 47%), mp 61~62°C (from MeOH), ¹H-NMR (CDCl_3 , 500 MHz) δ : 0.618 (3 H, s, 18-H₃), 0.941 (3 H, d, J = 6.56 Hz, 21-H₃), 0.943 (3H, s, 19-H₃), 1.095 (6 H, d x 2, J = 7.05 Hz, 26-H₃ and 27-H₃), 2.614 (1 H, sept, J = 7.05 Hz, 25-H), 3.640 (1 H, m, 3 α -H), 5.392 (1 H, dt, J = 5.59 and 2.80 Hz, 6-H), 5.576 (1 H, dd, J = 5.71 and 2.31 Hz, 7-H). EI-MS m/z (rel. int.) : 398 (M^+ , 75), 380 (42), 365 (100), 339 (39), 253 (54), 251 (35), 211 (31). HR-EI-MS; Calcd. for $\text{C}_{27}\text{H}_{42}\text{O}_2$ (M^+) : 398.3187, Found: 398.3179.

2·2·2 Preparation of ergosta-5,7,24(28)-trien-3 β -ol, 5-dehydroepisterol 3

A solution of 3 β -hydroxycholesta-5,7-dien-24-one 7 (61.0 mg, 0.153 mmol) in THF (6 mL) was treated with Tebbe reagent¹¹) (0.5 M, 0.6 mL, Aldrich) at 0°C under argon for 40 min. The work-up (diethyl ether) gave a crude product which was

Scheme 1 Reagents and conditions : i, *N*-bromosuccinimide, CCl_4 , reflux, 20 min; ii, $(n\text{-Bu})_4\text{NBr}$ /THF, room temp., 1 h; iii, $(n\text{-Bu})_4\text{NF}$ /THF, room temp., 30 min; iv, 5%KOH/MeOH, room temp., 2 h; v, Tebbe reagent/THF, 0°C, 40 min; vi, Na/*t*-BuOH-THF, 45°C, 2 h.

applied onto four plates of silica gel (20 cm x 20 cm, 0.5 mm thickness). The plates were developed twice with hexane-EtOAc (5 : 1, vol/vol). The silica gel band with R_F 0.26~0.33 was scraped off and eluted with EtOAc. Filtration and removal of the solvent *in vacuo* gave 5-dehydroepisterol **3** (28 mg, 46%), mp 118~120°C (from MeOH) (lit.⁸), mp 129~130.5°C), ¹H-NMR (CDCl₃, 500 MHz) δ : 0.620 (3 H, s, 18-H₃), 0.941 (3 H, s, 19-H₃), 0.970 (3 H, d, J =6.60 Hz, 21-H₃), 1.021 (3 H, d, J =6.86 Hz, 26-H₃), 1.026 (3 H, d, J =6.86 Hz, 27-H₃), 2.229 (1 H, sept, J =6.86 Hz, 25-H), 3.635 (1 H, m, 3 α -H), 4.657 (1 H, d, J =0.98 Hz, 28-H), 4.715 (1 H, s, 28-H), 5.386 (1 H, dt, J =5.84 and 2.43 Hz, 6-H), 5.571 (1 H, dd, J =5.46 and 2.41 Hz, 7-H). EI-MS m/z (rel. int.) : 396 (97), 381 (11), 378 (15), 363 (100), 349 (9), 337 (73), 323 (6), 253 (24), 251 (18), 211 (36). HR-EI-MS; Calcd. for C₂₈H₄₄O (M⁺) : 396.3394, Found : 396.3395.

2·2·3 Preparation of [26,27-²H₆] ergosta-5,7,24(28)-trien-3 β -ol, [26,27-²H₆] 5-dehydroepisterol **9**

As described above, [25,26,27-²H₇] 3 β -acetoxycholest-5-en-24-one **8** (37 mg), derived from a known [25,26,27-²H₇] 3 β -tetrahydropyranyloxycholest-5-en-24-one⁹), was converted to [26,27-²H₆] 5-dehydroepisterol **9** (7.5 mg), mp 119~122°C (from MeOH), ¹H-NMR (CDCl₃, 400 MHz) δ : 0.627 (3 H, s, 18-H₃), 0.947 (3 H, s, 19-H₃), 0.977 (3 H, d, J =6.83 Hz, 21-H₃), 2.195 (1H, br s, 25-H), 3.641 (1 H, m, 3 α -H), 4.661 (1 H, d, J =1.46 Hz, 28-H), 4.713 (1 H, s, 28-H), 5.392 (1 H, dt, J =5.86 and 2.44 Hz, 6-H), 5.576 (1 H, dd, J =5.37 and 2.44 Hz, 7-H). EI-MS m/z (rel. int.) : 402 (M⁺, 85), 387 (5), 384 (60), 369 (100), 343 (34), 253 (71), 251 (26), 211 (23). HR-EI-MS; Calcd. for C₂₈H₃₈OD₆ (M⁺) : 402.3770, Found : 402.3781.

2·2·4 Preparation of 5 α -ergosta-7,24(28)-dien-3 β -ol, episterol **4**

A solution of 5-dehydroepisterol **3** (14.0 mg, 35.4 μ mol) in *t*-butanol (1 mL) and THF (2 mL) was treated with sodium (five tips) at 45°C under argon for 2 h. The work-up (diethyl ether) gave a product (14 mg), a small portion of which was analyzed by GC-MS after trimethylsilylation, thus showing the product to consist of desired episterol **4** (24%) and starting material **3** (76%). By preparative HPLC [column, Senshu Pak ODS 4150-N (15 cm x 1.0 cm i.d.) ; mobile phase, MeOH ; flow rate, 2 mL·min⁻¹ ; detection, UV

205 nm], episterol **4** (1.1 mg) was isolated from one-half the mixture: mp 125~127°C (from MeOH) (lit.⁵), mp 125~126°C), ¹H-NMR (CDCl₃, 400 MHz) δ : 0.539 (3 H, s, 18-H₃), 0.797 (3H, s, 19-H₃), 0.955 (3H, d, J =6.83 Hz, 21-H₃), 1.023 (3H, d, J =6.83 Hz, 26-H₃), 1.029 (3 H, d, J =6.83 Hz, 27-H₃), 2.230 (1 H, sept, J =6.83 Hz, 25-H), 3.596 (1 H, m, 3 α -H), 4.658 (1 H, d, J =0.98 Hz, 28-H), 4.714 (1 H, s, 28-H), 5.162 (1 H, dd, J =4.88 and 2.43 Hz, 7-H). EI-MS m/z (rel. int.) : 398 (M⁺, 22), 383 (26), 380 (8), 365 (9), 314 (42), 299 (13), 271 (100), 269 (25), 255 (20), 253 (16), 231 (14), 213 (19). HR-EI-MS ; Calcd. for C₂₈H₄₆O (M⁺) : 398.3551, Found: 398.3549.

2·2·5 Preparation of [26,27-²H₆] 5 α -ergosta-7,24 (28)-dien-3 β -ol, [26,27-²H₆] episterol **10**

As described above, [26,27-²H₆] 5-dehydroepisterol **9** (4.5 mg) was converted to [26,27-²H₆] episterol **10** (1.68 mg), mp 126~129°C (from MeOH-chloroform), ¹H-NMR (CDCl₃, 400 MHz) δ : 0.541 (3 H, s, 18-H₃), 0.797 (3 H, s, 19-H₃), 0.955 (3 H, d, J =6.83 Hz, 21-H₃), 2.191 (1H, br s, 25-H), 3.590 (1 H, m, 3 α -H), 4.656 (1 H, d, J =0.97 Hz, 28-H), 4.707 (1 H, s, 28-H), 5.162 (1 H, dd, J =4.88 and 2.44 Hz, 7-H). EI-MS m/z (rel. int.) : 404 (M⁺, 27), 389 (23), 386 (21), 371 (14), 314 (51), 299 (11), 296 (17), 271 (100), 255 (21), 253 (37), 231 (11), 213 (18). HR-EI-MS ; Calcd. for C₂₈H₄₀OD₆ (M⁺) : 404.3927, Found : 404.3931.

3 Results and Discussion

Synthesis of 5-dehydroepisterol **3** and episterol **4** was initiated from a known 3 β -acetoxycholest-5-en-24-one **5**¹⁰). By the method used for vitamin D¹²), a 5,7-diene function was introduced as follows. Steroid **5** was brominated at the C-7 position with *N*-bromosuccinimide. The ratio of the resulting 7 α - to 7 β -bromides **6** increased by treatment with tetra-*n*-butylammonium bromide. The 7 α -bromide enriched mixture **6** was dehydrobrominated with tetra-*n*-butylammonium fluoride to afford, subsequent to saponification, 3 β -hydroxycholesta-5,7-dien-24-one **7** in 47% yield from **5**.

As reported for deuterio-labeled 24-methylenecholesterol and its related steroids⁹), the olefination of 3 β -hydroxycholesta-5,7-dien-24-one **7** was carried out with Tebbe reagent¹¹) and 5-dehydroepisterol **3** was obtained in 46% yield. Reduction of the 5,7-diene group of **3** with sodium

metal¹³⁾ gave a mixture of 5-dehydroepisterol **3** (76%) and episterol **4** (24%), from which episterol **4** was isolated by preparative HPLC.

Overall yields of 5-dehydroepisterol **3** and episterol **4** from **3** β -acetoxycholest-5-en-24-one **5** were low, compared to those obtained by Fryberg *et al.*⁸⁾. The present synthesis has the following merits : 1) milder and more rapid reaction conditions for dehydrobromination and olefination of 24-oxo group, 2) five-step reactions for the preparation of 5-dehydroepisterol **3** from **3** β -acetoxycholest-5-en-24-one **5** (nine steps from the same intermediate **5** including exchange of protection groups) and 3) one step preparation of episterol **4** from 5-dehydroepisterol **3** (multi-step preparation from ergosterol in their synthesis).

For the preparation of deuterio-labeled 5-dehydroepisterol and episterol, [25,26,27-²H₇] **3** β -acetoxycholesta-5,7-dien-24-one **8**, derived from a known [25,26,27-²H₇] **3** β -tetrahydropyranyloxycholesta-5,7-dien-24-one⁹⁾, was subjected to the above reaction steps, since a deuterium atom at the C-25 position was previously noted to be not lost during olefination of 24-oxo group with Tebbe reagent⁹⁾. In the present study, the deuterium atom at the C-25 position was completely lost possibly as a result of enolization during saponification. The products were [26,27-²H₆] 5-dehydroepisterol **9** and [26,27-²H₆] episterol **10**, whose d₆-content was found to be 98.5% by EI-MS. Nevertheless, as substrates in feeding experiment, d₆-sterols are sufficient for studying the biotransformation of episterol $\rightarrow$ 5-dehydroepisterol $\rightarrow$ 24-methylencholesterol in wild type and mutants of *Arabidopsis*.

The authors synthesized 5-dehydroepisterol **3**, episterol **4**, [26,27-²H₆] 5-dehydroepisterol **9** and [26,27-²H₆] episterol **10**. Clarification of the blocked biosynthetic steps of the *Arabidopsis* dwarf mutants, *dwf5* and *dwf7*, is presently in progress and the results will appear in a separate publication.

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〔報文〕 水分散系における各種トリアシルグリセリンの酸化安定性

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6種類のトリアシルグリセリン (TG) の水分散系での酸化安定性を、酸化に伴う高度不飽和脂肪酸 (PUFA) の減少量と過酸化物質生成量の測定から比較したところ、オリブ油 TG とマグロ眼窩油 TG の酸化安定性が最も高く、ついで、エゴマ油 TG、菜種油 TG、大豆油 TG、サフラワー油 TG の順となった。したがって、PUFA としては DHA や α -リノレン酸などの不飽和度のより高い PUFA を含む TG の方が、リノール酸のような不飽和度の低い PUFA を含む TG に比べ、酸化されにくいことがわかった。また、オレイン酸は、水分散系での TG の酸化に対して抑制的に働くことも明らかになった。次に、DHA 含量の異なる 5 種の TG の酸化安定性について検討したところ、DHA 含量が 30% 程度のものが最も酸化されにくかった。また、オレイン酸を TG 中に組み入れることでも、その酸化安定性の向上が期待できることがわかった。

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〔報文〕 ドコシルカラムを用いた HPLC による トリアシルグリセリン分子種分析の分離改善

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ドコシル (DCS) カラムを用いる逆相 HPLC 法によるトリアシルグリセリン (TG) 分子種分析について、その有効性を、保持比、分離係数、試料油分析を中心に、従来用いられてきたオクタデシル (ODS) カラムと比較、検討した。DCS カラムは ODS カラムに比べて TG の分離係数は大きく、TG の分離が良好であった。DCS カラムは ODS カラムに比べて低炭素鎖の TG に対しては保持力が弱い、長炭素鎖の TG に対しては保持力が強い。この差が TG 分子種分析の分離改善の要因になっていると考えられた。DCS カラムは試料油の大豆油等の植物油、さらには植物油に比べてより複雑な魚油、乳脂に対しても ODS カラムと比べてかなりの分離改善が認められ、多くの TG 分子種を良好に分離することが出来た。以上のことから DCS カラムは TG 分子種分析に対して有用なカラムの一つであると判別された。

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〔ノート〕 Episterol, 5-dehydroepisterol および それらの重水素標識体の合成

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シロイヌナズナの矮性ミュータントにおける内生ステロールの同定および代謝研究用に episterol, 5-dehydroepisterol およびそれらの重水素標識体 [26 , 27 - $^2\text{H}_6$] 5-dehydroepisterol, [26 , 27 - $^2\text{H}_6$] episterol を 3β -acetoxycholest-5-en-24-one またはその重水素標識体から、5, 7-ジエンの導入, 24-oxo 基の Tebbe 試薬によるオレフィン化および 5, 7-ジエンのナトリウムによる還元を鍵反応に用いて合成した。

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