Synthesis and Identification of a New Steroid, 24-Ethyl-5 α -cholest-24(28)*Z*-en-3-one in the Seeds of Foxtail Millet

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Abstract : Study was made to determine the structures of three minor 5 α -steroidal 3-ones detected in the seeds of foxtail millet (*Setaria italica* Beauv.). For this purpose, 24-ethyl-5 α -cholest-24(28)*Z*-en-3-one and its carbon-carbon double bond isomers were synthesized from isofucosterol. Based on direct GC-MS comparison with the synthetic samples, one steroid was found here for the first time to have the structure, 24-ethyl-5 α -cholest-24(28)*Z*-en-3-one. Two other minor steroids were identified as 24 ξ -ethyl-5 α -cholesta-7,22*E*-dien-3-one and 24 ξ -ethyl-5 α -cholest-7-en-3-one.

Key words : *Setaria italica* Beauv., 24-ethyl-5 α -cholest-24(28)*Z*-en-3-one, isofucosterol, 24 ξ -ethyl-5 α -cholesta-7,22*E*-dien-3-one, 24 ξ -ethyl-5 α -cholest-7-en-3-one

1 Introduction

In our investigation of brassinosteroid precursors, we recently reported the presence of seven steroidal 3-ones, viz., 24 ξ -methylcholest-4-en-3-one (**A**), 24 ξ -ethylcholest-4-en-3-one (**B**), 24 ξ -ethylcholesta-4,22*E*-dien-3-one (**C**), 24 ξ -methyl-5 α -cholestan-3-one (**D**), 24 ξ -ethyl-5 α -cholestan-3-one (**E**), 24 ξ -ethyl-5 α -cholest-22*E*-en-3-one (**F**) and 24-ethylcholesta-4,24(28)*Z*-dien-3-one (**G**) (Fig. 1), in the seeds of wheat (*Triticum aestivum* L.) and foxtail millet (*Setaria italica* Beauv.)¹⁾. In the seeds of foxtail millet, three additional minor steroids **X**₁, **X**₂ and **X**₃ were detected by GC-MS analysis, but their structures remained undetermined, because of the lack of authentic samples²⁾. In the mass spectrum (MS) of the steroid **X**₁, a molecular ion at *m/z* 412 was observed along with

ions at *m/z* 314, 299, 271, 258, 245, 244, 231, 229, 217, 203, 189, 175, 163, 149, 135, 123 and 109. The MS data suggest its structure to be 24-ethyl-5 α -cholestan-3-one with one double bond at the C-24(25), C-24(28)*E* or C-24(28)*Z* position, because a characteristic fragment ion due to McLafferty type rearrangement was observed at *m/z* 314 as a base peak. On the other hand, based on the reported MS data³⁾, the steroids **X**₂ and **X**₃ are likely to be 24 ξ -ethyl-5 α -cholest-7-en-3-one and 24 ξ -ethyl-5 α -cholesta-7,22*E*-dien-3-one, respectively.

To identify the steroid **X**₁, we synthesized at first a mixture of the above-mentioned possible double bond isomers, then synthesized the most plausible candidate and compared it with the natural product. For the identification of the steroids **X**₂ and **X**₃, we synthesized a mixture containing 24 ξ -ethyl-5 α -cholest-7-en-3-one and 24*S*-ethyl-5 α -cholesta-7,22*E*-dien-3-one from a 5 α - Δ^7 -sterol

Fig. 1 Structures of Steroidal 3-ones Found in the Seeds of Foxtail Millet.

fraction. In this paper, we report details of these syntheses and the identification of the minor steroids X_1 , X_2 and X_3 found in the seeds of foxtail millet.

2 Experimental

2.1 Analytical Methods

Melting points were determined under a hot-stage microscope (Yazawa micro melting point apparatus) and not corrected. $^1\text{H-NMR}$ spectra were recorded on a JEOL JMN-A400 (400 MHz) or a JEOL JMN-ECP500 (500 MHz) spectrometer in a deuterio-chloroform solution using tetramethylsilane as an internal standard. EI-MS and HR-EI-MS were measured with a JEOL SX-102 mass spectrometer at 70 eV.

2.2 GC-MS Analysis

GC-MS analysis was performed with a gas chromatograph-mass spectrometer (GC-MS QP-5000, Shimadzu Corporation) under the following condition: capillary column, DB-1 (30 m $\times$ 0.25 mm i.d., 0.25 μm film thickness, J & W Scientific); column temp., elevated from 270°C to 300°C at a

rate of 2°C/min, then 300°C for 5 min; injection, split; split ratio, 70 : 1; injection temp., 300°C, interface temp., 280°C; carrier gas, helium; carrier gas pressure, 100 kPa; total flow rate, 52.6 mL/min; column flow rate, 0.7 mL/min; ionization, electron impact (70 eV); scan interval, 0.5 sec.; mass range, m/z 100~550.

2.3 Synthesis of a Mixture Containing Possible Carbon-carbon Double Bond Isomers of the Steroid X_1

2.3.1 Preparation of 3 β -acetoxysteroid-5-en-24-one 2

A sterol fraction containing isofucosterol was obtained as its acetate from the broad bean pollen, as already reported⁹. The steryl acetate mixture (1.0 g) in tetrahydrofuran (THF) (20 mL) and H_2O (2 mL) was treated with osmium tetroxide (100 mg) and *N*-methylmorpholine *N*-oxide (1.20 g) at room temperature overnight. The work-up (ethyl acetate, EtOAc) gave a mixture of 24, 28-diols 1 (0.96 g), which in THF (15 mL) was treated with a solution of sodium periodate (1.02 g) in H_2O (5 mL) at room temperature for 2.5 h. The work-up

(EtOAc) and chromatography on a column of silica gel (2.0 cm i.d. $\times$ 25 cm, 70~230 mesh, Merck) with 4.8% EtOAc/hexane provided a known 3 β -acetoxycholest-5-en-24-one **2** (0.39 g), mp 117~118°C (from methanol, MeOH) [lit.⁹⁾, mp 128°C (solvent was not specified)]. Although our sample had lower melting point than the reported data, it was found to be a single compound by GC-MS. Its spectral data were as follows: ¹H-NMR (500 MHz) δ : 0.674 (3H, s, 18-H₃), 0.915 (3H, d, J =6.50 Hz, 21-H₃), 1.016 (3H, s, 19-H₃), 1.091 (6H, d $\times$ 2, J =6.83 Hz, 26-H₃ and 27-H₃), 2.033 (3H, s, acetyl), 2.610 (1H, sept, J =6.83 Hz, 25-H), 4.603 (1H, m, 3 α -H), 5.374 (1H, m, 6-H). EI-MS m/z (rel. int., %): 382 (M^+ -60, 100), 367 (15), 340 (3), 339 (3), 296 (14), 281 (6), 274 (6), 261 (11), 255 (16), 253 (7), 243 (3), 228 (6), 213 (16). HR-EI-MS; Calcd. for C₂₇H₄₂O (M^+ -60): 382.3238, Found: 382.3229.

2·3·2 Preparation of 3 β -hydroxycholest-5-en-24-one **3**

The steroid **2** (72 mg) in THF (5 mL) was treated with 5% KOH/MeOH (1 mL) at room temperature for 3 h. The work-up (chloroform) gave the title compound **3** (61 mg), mp 136~137°C (from MeOH), ¹H-NMR (500 MHz) δ : 0.676 (3H, s, 18-H₃), 0.915 (3H, d, J =6.52 Hz, 21-H₃), 1.007 (3H, s, 19-H₃), 1.090 (6H, d $\times$ 2, J =6.85 Hz, 26-H₃ and 27-H₃), 2.610 (1H, sept, J =6.85 Hz, 25-H), 3.521 (1H, m, 3 α -H), 5.352 (1H, m, 6-H). EI-MS m/z : 400 (M^+ , 100), 385 (19), 382 (63), 367 (27), 357 (7), 340 (7), 314 (45), 299 (18), 296 (11), 289 (27), 281 (15), 273 (13), 271 (23), 255 (28), 231 (15), 213 (41). HR-EI-MS; Calcd. for C₂₇H₄₄O₂ (M^+): 400.3343, Found: 400.3336.

2·3·3 Preparation of a mixture of fucosterol and its double bond isomers **6**

The steroid **3** (90 mg) in dichloromethane (CH₂Cl₂) (5 mL) was treated with 2,3-dihydropyran (0.5 mL) and *p*-toluenesulfonic acid monohydrate (5 mg) at room temperature for 1 h. The work-up (CH₂Cl₂) gave a tetrahydropyranyl ether **4** (91 mg), mp 133~134°C (from MeOH), which in THF (5 mL) was treated with *ca.* 3 *M* solution of ethylmagnesium bromide in diethyl ether (Et₂O) (1 mL, Tokyo Kasei) under argon at room temperature for 1 h. After the work-up (EtOAc), the resulting alcohol **5** in pyridine (3 mL) was treated with phosphorus oxychloride (0.5 mL) at room temperature for 3 d. After the work-up

(EtOAc), a product in THF (4 mL) and MeOH (2 mL) was treated with 2 *M* HCl (0.3 mL) at room temperature for 2 h. The work-up (EtOAc) and chromatography on a silica gel column (2.0 cm i.d. $\times$ 20 cm) with 17% EtOAc/hexane provided a sterol mixture of the title compounds **6** (50 mg).

A small portion of the mixture was analyzed by GC-MS after trimethylsilylation. The relative retention time (R_{tR}) of each sterol trimethylsilyl (TMS) ether to the t_R (10.57 min) of cholesterol TMS ether was determined and the composition was obtained by total ion chromatogram. The mixture was found to contain TMS ethers of 24-ethylcholesta-5,23*Z*-dien-3 β -ol (R_{tR} 1.25, 5.4%), 24-ethylcholesta-5,23*E*-dien-3 β -ol (R_{tR} 1.26, 10.0%), fucosterol (R_{tR} 1.29, 27.1%), isofucosterol (R_{tR} 1.31, 16.2%) and 24-ethyl-desmosterol (R_{tR} 1.36, 41.3%). The MS data of the former two were the same, m/z : 484 (M^+ , 8), 469 (7), 394 (7), 386 (7), 379 (10), 372 (8), 355 (11), 343 (51), 283 (68), 253 (40), 243 (12), 241 (20), 227 (12), 215 (23), 213 (16), 173 (34), 159 (53), 145 (42), 133 (100), 129 (96), 121 (39), 119 (61). The MS data of the latter three were the same, m/z : 484 (M^+ , 6), 469 (4), 394 (4), 386 (50), 379 (6), 371 (14), 355 (7), 343 (10), 296 (47), 281 (37), 257 (17), 255 (12), 253 (21), 227 (8), 213 (14), 211 (20), 173 (14), 161 (18), 159 (26), 147 (24), 145 (28), 133 (27), 129 (100), 121 (30), 119 (50).

The TMS ethers of fucosterol and isofucosterol were identified based on direct comparison with authentic samples (fucosterol was purchased from Wako and isofucosterol was obtained as described later). 24-Ethyl-desmosterol TMS ether was identified based on analysis of its MS data, which was superimposable to those of the TMS ethers of fucosterol and isofucosterol. The structure of each TMS ether of 24-ethylcholesta-5,23*Z*-dien-3 β -ol and 24-ethylcholesta-5,23*E*-dien-3 β -ol is based on both the MS data and the mechanism of the above-mentioned dehydration. The assignment of 23*E*- and 23*Z*-isomers is reversible and tentative.

2·3·4 Preparation of a mixture of 24-ethylcholesta-4,23*E*-, 4,23*Z*-, 4,24(28)*E*-, 4,24(28)*Z*- and 4,24(25)-dien-3-ones **7**

The sterol mixture **6** (50 mg) in 1-methyl-4-piperidone (1.4 mL) and toluene (5 mL) was treated with aluminum isopropoxide (0.15 g) at 110°C under argon for 1 h. After the work-up (EtOAc), a product was applied onto three plates

of silica gel (20 cm×20 cm, 0.5 mm thickness, Merck) and they were developed with 17% EtOAc/hexane. The silica gel band with R_F 0.30~0.37 was scraped off, eluted with EtOAc and concentrated to provide a mixture of the title compounds **7** (29 mg). The mixture was analyzed by GC-MS to contain 24-ethylcholesta-4, 23Z-dien-3-one (R_{tR} 1.36, 6.1%), 24-ethylcholesta-4, 23E-dien-3-one (R_{tR} 1.37, 8.3%), 24-ethylcholesta-4, 24(28)E-dien-3-one (R_{tR} 1.41, 29.3%), 24-ethylcholesta-4, 24(28)Z-dien-3-one (R_{tR} 1.43, 16.3%) and 24-ethylcholesta-4, 24(25)-dien-3-one (R_{tR} 1.48, 40.0%). The assignment of the former two is tentative and that of the latter three is based on the composition and the R_{tR} of the starting sterol mixture. The MS data of the former two were the same, m/z : 410 (M^+ , 37), 395 (7), 312 (11), 299 (51), 281 (54), 269 (100), 253 (10), 241 (7), 229 (13), 215 (10), 203 (21), 185 (20), 175 (56), 161 (47), 149 (96), 135 (52), 121 (55), 107 (64). The MS data of the latter three were the same, m/z : 410 (M^+ , 12), 395 (4), 313 (100), 312 (90), 297 (72), 283 (5), 268 (32), 256 (15), 243 (27), 231 (42), 227 (60), 201 (13), 189 (20), 173 (16), 159 (18), 147 (33), 133 (38), 124 (46), 105 (55).

2·3·5 Preparation of a mixture of 24-ethyl-5 α -cholest-23E-, 23Z-, 24(28)E-, 24(28)Z- and 24(25)-en-3-ones **8**

The enone mixture **7** (20 mg) in THF (5 mL) and ethanol (2 drops) was treated with a mixture of lithium (25 mg) and liquid ammonia (10 mL) at -78°C under argon for 1 h. Ammonium chloride (1.0 g) was added to the mixture and ammonia was evaporated at room temperature. After the work-up (EtOAc), a product was applied onto two plates of silica gel (20 cm×20 cm) and they were developed with 17% EtOAc/hexane. The silica gel band with R_F 0.43~0.49 was scraped off, eluted with EtOAc and concentrated to give a mixture of the title compounds **8** (10 mg). The mixture was analyzed by GC-MS to contain 24-ethyl-5 α -cholest-23Z-en-3-one (R_{tR} 1.23, 5.8%), 24-ethyl-5 α -cholest-23E-en-3-one (R_{tR} 1.24, 11.5%), 24-ethyl-5 α -cholest-24(28)E-en-3-one (R_{tR} 1.28, 26.3%), 24-ethyl-5 α -cholest-24(28)Z-en-3-one (R_{tR} 1.30, 21.5%) and 24-ethyl-5 α -cholest-24(25)-en-3-one (R_{tR} 1.35, 34.9%). The assignment of the former two is tentative and that of the latter three is based on the composition and the R_{tR} of the starting sterol mixture. The MS data of the former

two were the same, m/z : 412 (M^+ , 5), 397 (5), 314 (17), 301 (22), 299 (29), 283 (44), 271 (100), 257 (9), 255 (6), 245 (5), 231 (17), 217 (8), 215 (9), 205 (11), 191 (30), 187 (17), 177 (14), 175 (17), 163 (21), 161 (22), 159 (14), 151 (19), 149 (28), 147 (16), 145 (14), 137 (22), 135 (34), 133 (21), 123 (87), 121 (40), 109 (59), 107 (51). The MS data of the latter three were the same, m/z : 412 (M^+ , 3), 397 (1), 314 (100), 299 (42), 285 (4), 271 (21), 258 (10), 245 (12), 244 (10), 233 (11), 231 (23), 229 (14), 217 (12), 215 (7), 203 (7), 189 (6), 187 (5), 175 (7), 163 (13), 161 (13), 149 (13), 147 (11), 145 (10), 135 (21), 133 (11), 123 (25), 121 (26), 119 (14), 109 (28), 107 (28).

2·4 Isolation of Isofucoesterol **9**

The above-mentioned sterol acetate mixture³⁾ (0.57 g) in a minimum amount of 1% Et₂O/CH₂Cl₂ was applied onto a 10% silver nitrate (AgNO₃)-silica gel column (2.0 cm i.d.×33 cm) wrapped around in an aluminum foil. Elution was conducted with 1% Et₂O/CH₂Cl₂. Each fraction was collected in the amount of 20 mL and monitored by AgNO₃-TLC using 3% Et₂O/CH₂Cl₂ as a developing solvent. Fraction 6 (199 mg) was found to be a single spot by AgNO₃-TLC and determined by GC-MS to contain isofucoesterol acetate, mp 133~134°C (from MeOH) (lit.⁶⁾, mp 137~139°C). Its ¹H-NMR (500 MHz) and EI-MS data were identical with the reported data⁶⁾.

As described above, isofucoesterol acetate (110 mg) was saponified to afford isofucoesterol **9** (98 mg), mp 134~135°C (from MeOH) (lit.⁶⁾, mp 139~140°C). Its ¹H-NMR (500 MHz) and MS data were identical with the reported data⁶⁾.

2·5 Synthesis of 24-ethyl-5 α -cholest-24(28)Z-en-3-one **11**

As described above, isofucoesterol **9** (44 mg) was subjected to Oppenauer oxidation to afford 24-ethylcholesta-4, 24(28)Z-dien-3-one **10** (19 mg) : mp 66~68°C (from MeOH), ¹H-NMR (400 MHz) δ : 0.714 (3H, s, 18-H₃), 0.943 (3H, d, $J=6.84$ Hz, 21-H₃), 0.976 (6H, d×2, $J=7.33$ Hz, 26-H₃ and 27-H₃), 1.182 (3H, s, 19-H₃), 1.591 (3H, d, $J=6.84$ Hz, 29-H₃), 2.831 (1H, sept, $J=6.84$ Hz, 25-H), 5.105 (1H, q, $J=6.84$ Hz, 28-H), 5.724 (1H, s, 4-H). Its MS data by GC-MS was the same as described above. HR-EI-MS ; Calcd. for C₂₉H₄₆O (M^+) : 410.3551, Found : 410.3553.

As described above, the steroid **10** (15 mg) was subjected to Birch reduction to give 24-ethyl-5 α -

cholest-24(28)*Z*-en-3-one **11** (9.5 mg) : mp 151~151.5°C (from MeOH), ¹H-NMR (400 MHz) δ : 0.681 (3H, s, 18-H₃), 0.935 (3H, d, J =6.34 Hz, 21-H₃), 0.974 (6H, d $\times$ 2, J =6.83 Hz, 26-H₃ and 27-H₃), 1.007 (3H, s, 19-H₃), 1.589 (3H, d, J =6.83 Hz, 29-H₃), 2.829 (1H, sept, J =6.83 Hz, 25-H), 5.104 (1H, q, J =6.83 Hz, 28-H). Its MS data by GC-MS was the same as described above. HR-EI-MS; Calcd. for C₂₉H₄₈O (M⁺) : 412.3707, Found : 412.3711.

2·6 Preparation of a Mixture Containing 24*S*-ethyl-5 α -cholesta-7,22*E*-dien-3-one and 24 ξ -ethyl-5 α -cholest-7-en-3-one

As previously described⁷⁾, a 5 α - Δ^7 -sterol fraction containing spinasterol and 24 ξ -ethyl-5 α -cholest-7-en-3 β -ol was obtained from the *Amaranthus* seeds. The sterol fraction (12 mg) in acetone (4 mL) was treated with Jones reagent (5 drops) at room temperature for 20 min. The work-up (EtOAc) gave a mixture of 5 α - Δ^7 -steroidal 3-ones (11 mg). The R_{tR} and MS data of 24*S*-Ethyl-5 α -cholesta-7,22*E*-dien-3-one by GC-MS were as follows : R_{tR} 1.23, EI-MS m/z : 410 (M⁺, 9), 395 (2), 381 (0.5), 367 (9), 349 (0.5), 325 (1), 312 (2), 298 (14), 283 (5), 269 (100), 257 (6), 255 (6), 253 (4), 244 (7), 229 (17), 217 (4), 213 (7), 201 (4), 187 (6), 185 (5), 175 (8), 173 (8), 161 (16), 159 (12), 149 (11), 147 (24), 137 (15), 135 (14), 123 (24), 121 (15), 119 (23), 109 (24), 107 (22), 105 (33). The R_{tR} and MS data of 24 ξ -ethyl-5 α -cholest-7-en-3-one by GC-MS were as follows : R_{tR} 1.33, EI-MS m/z : 412 (M⁺, 68), 397 (33), 299 (5), 271 (100), 258 (14), 253 (11), 244 (30), 229 (63), 227 (9), 216 (11), 213 (36), 211 (12), 201 (20), 189 (9), 187 (19), 185 (10), 175 (14), 173 (18), 171 (22), 161 (40), 159 (23), 149 (23), 147 (36), 145 (21), 135 (41), 133 (30), 131 (26), 123 (36), 121 (43), 119 (62), 111 (31), 109 (32), 107 (50), 105 (67).

2·7 Identification of the Steroids X₁, X₂ and X₃ in the Seeds of Foxtail Millet

As previously described¹⁾, a 5 α -steroidal 3-one fraction was obtained from the seeds of foxtail millet. By use of the synthetic samples as standards, this fraction was reanalyzed by GC-MS.

3 Results and Discussion

To identify the steroid X₁, a mixture of possible double bond isomers at the C-24(25), C-24(28)*E* and C-24(28)*Z* position was at first synthesized (Scheme 1). The steryl acetate mixture⁴⁾ was

dihydroxylated and the resulting mixture of vicinal diols **1**, without separation, was oxidatively cleaved to provide a known 3 β -acetoxycholest-5-en-24-one **2**⁵⁾. After exchange of the protecting group of **2**, the tetrahydropyranyl ether **4** was reacted with ethylmagnesium bromide, dehydrated with phosphorous oxychloride and deprotected to afford a sterol mixture **6**. The mixture was subjected to Oppenauer oxidation to give a mixture of steroidal 4-en-3-ones **7**. It is well known that the Birch reduction of steroidal 4-en-3-ones gives thermodynamically stable 5 α -steroidal 3-ones⁸⁾. Thus, the steroidal 4-en-3-ones **7** was subjected to Birch reduction to provide a mixture of 5 α -steroidal 3-ones **8**. The R_{tR} and MS data of the steroidal 4-en-3-ones **7** and the 5 α -steroidal 3-ones **8** are summarized in **Experimental** section.

Having the mixture of the double bond isomers in hand, we reanalyzed the 5 α -steroidal 3-one fraction¹⁾ from the seeds of foxtail millet by GC-MS. As expected, the MS data of the steroid X₁ was in agreement with those of the C-24(25), C-24(28)*E* and C-24(28)*Z* double bond isomers, but not with those of the C-23(24) double bond isomers. Among the former three isomers, the least component had the same R_{tR} as the natural product X₁ (R_{tR} 1.30), suggesting that the steroid X₁ is a C-24(28)*Z* isomer, because among the sterol mixture **6**, the least component with C-24(28) double bond was found to be isofucosterol. Further, it is already found that its structurally-related steroid, 24-ethylcholesta-4,24(28)*Z*-dien-3-one **10** occurs in the seeds¹⁾. The steroid X₁ is most likely to be 24-ethyl-5 α -cholest-24(28)*Z*-en-3-one.

To synthesize this putative steroid, isofucosterol **9** is an ideal starting material, because it has the same carbon skeleton including the *Z* configuration of 24-ethylidene moiety as the target steroid. Oppenauer oxidation of isofucosterol **9** gave 24-ethylcholesta-4,24(28)*Z*-dien-3-one **10**, which was subjected to Birch reduction to afford 24-ethyl-5 α -cholest-24(28)*Z*-en-3-one **11** (Scheme 1). The *Z*-configuration of the steroid **11** was confirmed by a characteristic chemical shift of the proton at the C-25 position (δ 2.829, sept, J =6.83 Hz), which is in good agreement with that (δ 2.831, sept, J =6.9 Hz) of isofucosterol, but not with that (δ 2.198, sept, J =6.9 Hz) of its *E*-isomer, fucosterol⁶⁾. To confirm the 5 α -configuration of the steroid **11**, the steroid **11** was hydrogenated and the resulting

Scheme 1 Reagents and conditions : i, OsO₄, *N*-methylmorpholine *N*-oxide, THF-H₂O, room temp., overnight, ii, NaIO₄, THF-H₂O, room temp., 2.5 h ; iii, 5% KOH/MeOH, THF, room temp., 1 h ; iv, 2, 3-dihydropyran, *p*-TsOH-H₂O, CH₂Cl₂, room temp., 1 h ; v, EtMgBr, THF, room temp., 1 h ; vi, POCl₃, pyridine, room temp., 3 d ; vii, 2*M* HCl, THF-MeOH, room temp., 2 h ; viii, Al(*i*-PrO)₃, 1-methyl-4-piperidone, toluene, 110°C, 1 h ; ix, Li, liq. NH₃-EtOH/THF, -78°C, 1 h.

product was analyzed by GC-MS to be identical with 24-ethyl-5 α -cholestan-3-one in terms of t_R and MS data, which was derived from sitostanol by Jones oxidation²⁾. Discrimination between 5 α - and 5 β -steroidal 3-ones by MS data is already described in a literature³⁾. Thus, the structure of the steroid **11** was unambiguously confirmed.

GC-MS analysis indicated that the steroid **X₁** with R_{tR} 1.30 was identical with 24-ethyl-5 α -cholest-24(28)*Z*-en-3-one **11** in terms of t_R and MS data. To the best of our knowledge, the present work is the first identification of the steroid **11**. In foxtail millet, this steroid is assumed to be derived from 24-ethylcholesta-4,24(28)*Z*-dien-3-one **10** by reduction of the C-4 double bond, as it is already demonstrated in *Arabidopsis thaliana* L.^{9),10)} and *Catharanthus roseus*¹¹⁾ that in an early part of a brassinolide biosynthesis, (24*R*)-24-methyl-5 α -

cholestan-3-one is produced by enzymatic reduction of (24*R*)-24-methylcholest-4-en-3-one. Two steroids **X₂** and **X₃** with R_{tR} 1.23 and 1.33 were identified as 24 ξ -ethyl-5 α -cholesta-7, 22*E*-dien-3-one and 24 ξ -ethyl-5 α -cholest-7-en-3-one, respectively, based on direct GC-MS comparison with the synthetic samples. These two steroids were already found present in the herb *Verbascum thapsus*³⁾.

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[ノート] アワ種子中に含まれる新規ステロイド,
24-Ethyl-5 α -cholest-24(28) Z-en-3-one の合成と同定

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アワ (*Setaria italica* Beauv.) 種子中から検出された3種の微量な5 α -ステロイド系3-オンの構造決定を行った。これらを同定するために、24-ethyl-5 α -cholest-24(28) Z-en-3-one およびその炭素-炭素二重結合異性体をイソフコステロールから合成した。合成標品とのGC-MSによる直接比較により、一つを24-ethyl-5 α -cholest-24(28) Z-en-3-one とはじめて同定した。さらに、残りの2種も24 ξ -ethyl-5 α -cholesta-7,22E-dien-3-one および24 ξ -ethyl-5 α -cholest-7-en-3-one と同定した。

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