Phytochemistry, 1975, Vol. 14, pp. 529–532. Pergamon Press. Printed in England.

STEROIDAL CONSTITUENTS OF *SOLANUM XANTHOCARPUM*

GENJIRO KUSANO and TSUNEMATSU TAKEMOTO

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai, Japan

and

JOHN A. BEISLER and YOSHIO SATO

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Services, U.S. Department of Health, Education and Welfare, Bethesda, MD, U.S.A.

(*Received* 17 *June* 1974)

Key Word Index—*Solanum xanthocarpum*; Solanaceae; steroids; carpesterol derivatives; synthesis from carpesterol.

Abstract—From the extract of the fruits of *Solanum xanthocarpum* (Solanaceae), five new steroidal compounds were isolated and characterized: 4α-methyl-24ξ-ethyl-5α-cholest-7-en-3β,22ξ-diol (**1**), 3β,22ξ-dihydroxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one (**2**), 3β-benzoxy-14β,22ξ-dihydroxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one (**3**), 3β-benzoxy-14α,22ξ-dihydroxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one (**4**) and 3β-(*p*-hydroxy)-benzoxy-22ξ-hydroxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one (**5**).

INTRODUCTION

As a result of our continuing investigation of the constituents of *Solanum xanthocarpum*, from which carpesterol (**7**) [1,2] and 4α-methyl-24-ethylcholest-7-en-3β-ol (**6**) [3] have been isolated and characterized, we would like to report the isolation of five additional new steroids (**1–5**) along with the proof of their structural assignments.

A comparison of the spectroscopic data for **1–5** with the corresponding data for carpesterol (**7**) and 4α-methyl-24-ethylcholest-7-en-3β-ol (**6**) provided us initially with very strong indications as to the structural identity of the five most recently isolated compounds. However, since the structure and absolute stereochemistry of **7** have been firmly established [2], and since useful quantities of **7** were on hand to serve as starting material, it was possible to identify more thoroughly compounds (**1–5**) by their synthesis.

RESULTS AND DISCUSSION

The MS of compounds **1–7** are presented in Table 1. It can be clearly seen that **1** gave the parent peak at *m/e* 444 ($C_{30}H_{52}O_2$) and that both **1** and **6** give strong peaks in their MS at *m/e* 287 and 245 that are characteristic of steroids with an isooctyl side chain [4]. Moreover, **1** and **6** both produced ions at *m/e* 285 and 243 indicative of Δ^7-steroids [5]. Also in the MS of **1** there was an ion at *m/e* 316 arising from a rearrangement on electron impact which has been commonly observed [6] in the spectra of 22-hydroxysteroids. A doublet at 3·74 ppm in the NMR spectrum of **1** can be also recognized in the NMR spectrum of **7**. Thus, it would seem that **1** and **7** probably have an identical side chain stereochemistry (*i.e.* 22*R*-OH and 24*R*-ethyl). Carpesterol (**7**) was subjected to a modified Wolf–Kishner reduction [7], which produced two products, **1** and **8**. The product (**1**) was shown to be identical to the natural product (**1**) by comparison of melting point, $[\alpha]_D$, IR, NMR, and MS. The second product (**8**) was assumed to be the $\Delta^{8(14)}$-isomer of **1** because no vinyl proton appeared in the NMR spectrum, and because it was known that the Δ^7-double bond of carpesterol (**7**) readily isomerizes to the $\Delta^{8(14)}$-position under alkaline conditions.

For the purpose of identifying compound **2** (debenzoylcarpesterol), carpesterol (**7**) was hydrolysed with 2% K_2CO_3 to give the debenzoyl derivative which was identical to **2** in m.p. $[\alpha]_D$, IR,

Table 1. MS of the steroids of *S. xanthocarpum*

	M⁺	M⁺-R_1OH	*a*	*a*-R_1OH	*a*-R_1OH -Me	*b*	*b*-2H	*b*-R_1OH	*c*	*c*-R_1OH	*d*	*d*-2H	*d*-R_1OH	Others
(1)	444 (51)	426 (19)	316 (49)			287 (65)	285 (65)	269 (76)			245 (40)	243 (22)	227 (46)	302, 219, 191 (100) (49) (87)
(2)	458 (22)	440 (51)	330 (100)		297 (28)				275 (96)	257 (52)				
(3)	578 (05)													560 (M⁺-18,05), 542 (66·7), 438 (30), 420 (100)
(4)	578 (1)													438, 402, 280 (49) (100) (28)
(5)	578 (18)	440 (67)	450 (7)	312 (66)	297 (36)			283 (23)		257 (100)				
(6)	428 (100)	410 (9)				287 (37)	285 (49)	269 (49)			245 (30)	243 (18)	227 (35)	
(7)	562	440	434	312	297			283		257				

Intensities relative to the base peak are given in brackets.

NMR and MS. An isomer **9** of **2** was also isolated as well as **10** an isomer of carpesterol **7.**

Compounds 3 and 4 showed weak parent peaks in the MS similar to the 14-hydroxy substituted ecdysterones [8]. Relative to the broadened singlet due to the carpesterol C-7 proton, the protons of 3 and 4 experienced a down field shift in the NMR spectra accompanied by a resolution of the signal to a doublet (J 3 Hz) indicating the presence of a 14-hydroxy group. Moreover, the observed down field shift (0·15 ppm for **3** and 0·09 ppm for **4**) of the C-13 methyl group singlets of **3** and **4** relative to that of carpesterol (**7**) can be reasonably explained if a 14β-hydroxyl group for **3** and 14β-hydroxyl group for **4** are present [9]. Carpesterol (**7**) on SeO_2 oxidation afforded **4** along with small quantities of the isomeric 14β-hydroxy derivative (**3**). Because **3** and 4 showed no change under SeO_2 treatment for a longer time, the possibility was excluded that **3** was a 9-hydroxy derivative.

In order to verify the structure of **5** by synthesis a circuitous route was necessary because of the small amount of **5** at our disposal. Thus, carpesterol (**7**) was converted to the acetate which was selectively hydrolyzed with 2% K_2CO_3 to liberate the 3β-hydroxyl function. The resulting debenzoyl compound (**11**) was separated from its $\Delta^{8(14)}$ isomer (**12**) via SiO_2 chromatography and treated with *p*-acetoxybenzoyl chloride in pyridine to give the *p*-acetoxybenzoyl derivative (**5**), which was identical to the acetate (**5a**) of the naturally occurring compound [10].

From the comparison of the spectral properties of the isolated sterols (**1–5**) with those of **6** and **7**, and the synthesis of compounds (**1–5**) from carpesterol (**7**), it follows that the five new steroids from *S. xanthocarpum* have structures **1–5**. However the stereochemistry of some asymmetric centers, especially at C-22 and C-24 is still somewhat in doubt because the methods so far employed (mp. $[\alpha]_D$, IR, NMR and MS) in our study are not completely adequate for unambiguous stereochemical designations at C-22 and C-24.

EXPERIMENTAL

Isolation process. After the ppt. (92 g) of crude carpesterol was filtered from the ligroin extract of the dried fruits (74 kg) of *Solanum xanthocarpum*, 15 l. of the filtrate was chromatographed on Al_2O_3 (3 kg). The column was eluted with hexane and C_6H_6 (Fraction A), C_6H_6–AcOEt (4:1 v/v) (Fraction B) and C_6H_6–AcOEt (1:1 v/v) (Fraction C). After crude carpesterol was obtained as a crystalline material from Fraction A, the soln was combined with Fraction B. The resulting A–B fraction (50 g) was chromatographed on SiO_2 (200 g) and eluted with C_6H_6 (Fraction D), C_6H_6–AcOEt (19:1 v/v) (Fraction E), C_6H_6–AcOEt (9:1 v/v) (Fraction F), C_6H_6–AcOEt (4 ~ 3:1 v/v) (Fraction G) and AcOEt. Fraction E (2·8 g) was chromatographed on SiO_2–$AgNO_3$ (10%, 20 g). The hexane–C_6H_6 (1:2 v/v) eluate gave crude carpesterol; the C_6H_6 eluate gave **1** (0·14 g).

After the ligroin extraction, the residual dried fruit was extracted with hot ethanol. The portion of the ethanol extracted material which was soluble (187 g) in AcOEt–MeOH (4:1 v/v) was chromatographed on Al_2O_3 (1 kg) and eluted successively with AcOEt–MeOH (4:1 v/v) (Fraction H), AcOEt–MeOH (2 ~ 1:1 v/v) and MeOH. Fraction H (9·2 g) was again chromatographed on Al_2O_3 (100 g). The C_6H_6–AcOEt (10 ~ 0:0 ~ 10 v/v) eluates (8 g) were further chromatographed on SiO_2 (150 g), whereupon the C_6H_6–Et_2O (9:1 v/v) eluate gave crude carpesterol (0·25 g), the C_6H_6–AcOEt (20:1 v/v) eluate gave **5** (12 mg), C_6H_6–AcOEt (10:1 v/v) eluted **1** (31 mg) and C_6H_6–AcOEt (2:1 v/v) eluted **2** (20 mg).

On TLC the crude carpesterol showed 4 spots besides carpesterol after repeated development. Thus, crude carpesterol (3 g) was fractionated via preparative TLC [silica gel GF, precoated; Analtech, Inc. Newark, Delaware U.S.A.; C_6H_6–AcOEt (10:1 v/v)] by repeating the development of the plate 3 times. The

first band was pure carpesterol (**7**, 2·4 g), the second was a mixture (0·46 g) of carpesterol and 4-desmethylcarpesterol (unpublished results), the third was **3** (27 mg), the fourth was **4** (8 mg) and the slowest was **5** (39 mg).

4α-methyl-24ξ-ethyl-5α-cholest-7-en-3β,22ξ-diol. (**1**). Recrystallization from Me_2CO, needles, m.p. 180–181°, $[\alpha]_D^{20}$ + 19·5°. (Found: C, 80·99; H, 11·72. $C_{30}H_{52}O_2$ requires: C, 81·02; H, 11·79%). IR ($CHCl_3$) cm^{-1}: 3600, 3450 (OH). NMR ($CDCl_3$) δ: 3·18 ppm (1H, *m*, C-3α), 5·18 (1H, *bd*, *J* 6 Hz, C-7), 3·73 (1H, *d*, *J* 12 Hz), 0·57 (3H, *s*, C-18), 0·83 (3H, *s*, C-19).

Modified Wolf–Kishner reduction of carpesterol (**7**). Capesterol (481 mg) was heated with hydrazine (1·9 g) and hydrazine dihydrochloride (681 mg) in triethylene glycol (10 ml) for 4 hr at 180°. The condenser was removed, pellets of KOH (2 g) added and the temp. was increased to 210° for 2 hr. After cooling, H_2O was added and the mixture was extracted with AcOEt. The AcOEt layer was washed with H_2O and dried over Na_2SO_4. The residue was separated on preparative TLC (C_6H_6–AcOEt (10 : 1 v/v), developed 3 times). The fastest running band was 173 mg of **1**, m.p. 181°, $[\alpha]_D^{20}$ + 19·5°. The IR and NMR spectra were superimposable with the corresponding spectra of the isolated, naturally occurring sterol (**1**). The remaining band yielded 92 mg of **8**, m.p. 171–172°, $[\alpha]_D^{20}$ + 26·8°. (Found: C, 81·00; H, 11·66. $C_{30}H_{52}O_2$ requires: C, 81·02; H, 11·79%). IR ($CHCl_3$) cm^{-1}: 3600, 3450 (OH). NMR ($CDCl_3$) δ: 3·17 ppm (1H, *m*, C-3α), 3·73 (1H, *d*, *J* 12 Hz), 0·63 (3H, *s*, C-18), 0·79 (3H, *s*, C-19).

3β,22ξ-Dihydroxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one (**2**). Recrystallized from aq. Me_2CO, m.p. 188–190°, $[\alpha]_D^{20}$ + 14·3°. (Found: C, 78·52; H, 10·90. $C_{30}H_{50}O_3$ requires: C, 78·55; H, 10·99%). IR ($CHCl_3$) cm^{-1}: 3600, 3510 (OH), 1668 (α,β-unsaturated carbonyl), 1625 (double bond). NMR ($CDCl_3$) δ: 5·68 ppm (1H, *bs* C-7), 3·74 (1H, *d*, *J* 12 Hz, C-22), 3·18 (1H, *m*, C-3α), 1·16 (3H, *d*, *J* 6 Hz, C-30), 0·86 (3H, *s*, C-19), 0·61 (3H, *s*, C-18). MS: *m/e* (relative intensity), 458 (22·2), 440 (51·1), 330 (100·0), 275 (95·6), 257 (52·2), 297 (27·8).

Hydrolysis of carpesterol (**7**). **7** (104 mg) was dissolved in 2% (w/v) K_2CO_3 in aq. MeOH and stirred for one day. H_2O was added, and the ppt. was filtered and dried. The three products of the reaction were separated using preparative TLC. Compound **2**, m.p. 188–190°, $[\alpha]_D^{20}$ + 14·4°, the IR and NMR spectra were superimposable with those of the isolated natural product (**2**). Compound **9** m.p. 164–165°, $[\alpha]_D^{20}$ + 22·5°. (Found: C, 78·52; H, 10·72. $C_{30}H_{50}O_3$ requires: C, 78·55; H, 10·99%). IR ($CHCl_3$): 3600, 3510 (OH), 1710 (Keto group). Compound **10**, m.p. 191–192°, $[\alpha]_D^{20}$ + 46·6°. (Found: C, 78·85; H, 9·49. $C_{37}H_{54}O_4$ requires: C, 78·96; H, 9·67%).

3β-Benzoxy-14β,22ξ-dihydroxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one (**3**). Recrystallized from AcOEt, m.p. 277–278°, $[\alpha]_D^{20}$ + 82·2°. (Found: C, 76·79; H, 9·31. $C_{37}H_{54}O_5$ requires: C, 76·77; H, 9·40%). IR ($CHCl_3$) cm^{-1}: 3600, 3500 (OH), 1710 (ester), 1670 (α,β-unsaturated carbonyl), 1625 (aromatic). NMR ($CDCl_3$) δ: 8·06 ppm (2H, *q*, J_1 7, J_2 1·5 Hz), 7·56 (1H, *q*, J_1 7, J_2 1·5), 7·41 (2H, *t*, J_1 7 J_2 7), 5·91 (1H, *d*, *J* 3 Hz, C-7), 4·70 (1H, *m*, C-3α), 3·75 (1H, *d*, *J* 12 Hz, C-22), 1·16 (3H, *d*, *J* 6 Hz, C-30), 0·93 (3H, *s*, C-19), 0·77 (3H, *s*, C-18). MS: *m/e* (relative intensity), 578 (0·5), 560 (0·5), 542 (66·7), 438 (30·0), 420 (100).

3β-Benzoxy-14α,22ξ-dihydroxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one (**4**). Recrystallized from AcOEt, m.p. 268–270°, $[\alpha]_D^{20}$ + 100·7°. (Found: C, 76·55; H, 9·21. $C_{37}H_{54}O_5$ requires: C, 76·77; H, 9·40%). IR ($CHCl_3$) cm^{-1}: 3600, 3450 (OH), 1712 (ester), 1680 (α,β-unsaturated keto group), 1631 (aromatic). NMR ($CDCl_3$) δ: 8·06 ppm (2H, *q*, J_1 7, J_2 1·5), 7·56 (1H, *q*, J_1 7, J_2 1·5), 7·41 (2H, *t*, J_1 7, J_2 7), 5·91 (1H, *d*, *J* = 3, C-7), 4·70 (1H, *m*, C-3α), 3·74 (1H, *d*, *J* 12, C-22), 1·16 (3H, *d*, *J* 6, C-30), 0·93 (3H, *s*, C-19), 0·71 (3H, *s*, C-18).

(22R,24R)-3β-Benzoxy-14β,22-dihydroxy-4α-methyl-24-ethyl-5α-cholest-7-en-6-one (**3**) *and (22R,24R)-3β-Benzoxy-14α,22-dihydroxy-4α-methyl-24-ethyl-5α-cholest-7-en-6-one* (**4**) *from carpesterol* (**7**). **7** (156 mg) was dissolved in dixoane (10 ml), ScO_2 (57 mg) added to the soln, and the mixture heated on the steam bath under N_2 for 1 hr. The inorganic ppt. was removed by filtration and the clear solution diluted with H_2O (20 ml) and allowed to stand overnight. The ppt. was filtered, washed with H_2O and dried. Because TLC showed two products (ratio, 9 : 1), separation was undertaken by preparative TLC [C_6H_6–AcOEt (10 : 1 v/v)]. The fastest running band gave **3** (17 mg, m.p. 277–278°, $[\alpha]_D^{20}$ + 82·0°) and the slower band was **4** (114 mg, m.p. 268–270°, $[\alpha]_D^{20}$ + 100·9°). IR and NMR spectra were superimposable with those of the isolated compounds (**3** and **4**).

3β-(p-Hydroxy)benzoxy-22ξ-hydroxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one (**5**). Recrystallized, from AcOEt, m.p. 294–296°, $[\alpha]_D^{20}$ + 71·9°. IR (Nujol) cm^{-1}: 3480, 3250 (OH), 1671 (ester + α,β-unsaturated keto group), 1625 (aromatic). IR ($CHCl_3$) cm^{-1} : 3600 (OH), 1671 (ester + α,β-unsaturated keto group), 1625 (aromatic). (Found: C, 76·74; H, 9·28. $C_{37}H_{54}O_5$ requires: C, 76·77; H, 9·40%). MS: *m/e* (relative intensity), 578 (17·9), 440 (67·0), 450 (7·1), 312 (66·1), 283 (22·3), 257 (100·0), 297 (35·7).

3β-(p-Acetoxy)benzoxy-22ξ-acetoxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one (**5a**). **5** (29 mg) was acetylated with Ac_2O in pyridine. The product was purified through Al_2O_3 chromatography. C_6H_6 eluate gave the acetate (**5a**), recrystallized from Me_2CO, m.p. 277–278°, $[\alpha]_D^{20}$ + 89·2°. (Found: C, 74·11; H, 8·77. $C_{41}H_{58}O_7$ requires: C, 74·28; H, 8·82%). IR ($CHCl_3$) cm^{-1}: 1710 (acetate), 1671 (ester + α,β-unsaturated keto group), 1625 (aromatic). NMR ($CDCl_3$) δ: 8·13 (2H, *d*, *J* 9 Hz), 7·21 (2H, *d*, *J* 9), 5·07 (1H, *d*, *J* 12 Hz, C-22), 5·73 (1H, *bs*, C-7), 4·91 (1H, *m*, C-3α), 2·04 (3H, *s*, $COCH_3$), 2·33 (3H, *s*, $COCH_3$), 0·93 (3H, *s*, C-19), 0·62 (3H, *s*, C-18).

Carpesteryl acetate (**7**). Carpesterol (189 mg) was acetyled with Ac_2O (0·2 ml) in pyridine (3 ml) and recrystallized from Me_2CO, m.p. 266–267°, $[\alpha]_D^{20}$ + 80·2°. IR ($CHCl_3$) cm^{-1}: 1725, 1710 (acetyl, benzoyl ester), 1680 (α,β-unsaturated keto group). NMR ($CDCl_3$) δ: 8·08 (2H, *q*, J_1 2 Hz, J_2 7·5), 7·5 (3H, *m*), 5·70 (1H, *bs*, C-7), 5·07 (1H, *d*, *J* 11 Hz, C-22), 4·70 (1H, *m*, C-3), 0·62 (3H, *s*, C-18), 0·93 (3H, *s*, C-19).

Hydrolysis of carpesteryl acetate (**7a**). The acetate (96·5 mg) was dissolved in ml aq. MeOH (MeOH : H_2O 9 : 1 v/v) containing K_2CO_3 (100 mg), and the mixture was refluxed for 1·5 hr. H_2O was added and the mixture was warmed gently on a steam bath for 30 min. After cooling the ppt. was filtered, dried and chromatographed on SiO_2 (20 g). The C_6H_6–AcOEt (10 : 1 v/v) eluate gave the debenzoyl derivative (**11**, 50 mg) of carpesteryl acetate and the isomer (**12**, 18 mg). Compound **11** was recrystallized from Me_2CO, m.p. 192–193°, IR ($CHCl_3$) cm^{-1}: 3595, 3540 (OH), 1720–1710 (acetyl). NMR ($CDCl_3$) δ: 5·06 (1H, *d*, *J* 11 Hz, C-22), 2·04 (3H, *s*, $COCH_3$), 0·84 (3H, *s*, C-19), 0·63 (3H, *s*, C-18).

(22R,24R)-3β-(p-Acetoxy)benzoxy-22-acetoxy-4α-methyl-24-ethyl-5α-cholest-7-en-6-one (**5a**). **11** (31 mg) was dissolved in pyridine (3 ml) and *p*-acetoxyl benzoyl chloride (2 drops) was added to the soln. After standing at room temp. overnight, H_2O was added and the ppt. was filtered, washed with H_2O dried and recrystallized from EtOH, m.p. 278–279°, $[\alpha]_D^{20}$ + 87·4°. The IR and NMR specta were superimposable with those of the diacetate of the nautrally occurring compound **5**.

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