Pergamon

PII: S0031-9422(97)01107-2

Phytochemistry, Vol. 48, No. 2, pp. 301–305, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

SASANQUOL, A 3,4-*seco*-TRITERPENE ALCOHOL FROM SASANQUA OIL, AND ITS ANTI-INFLAMMATORY EFFECT

TOSHIHIRO AKIHISA,* KEN YASUKAWA,† YUMIKO KIMURA,† SAKAE YAMANOUCHI† and TOSHITAKE TAMURA

College of Science and Technology, Nihon University, 1–8, Kanda Surugadai, Chiyoda-ku, Tokyo 101, Japan; † College of Pharmacy, Nihon University, 7-7-1, Narashinodai, Funabashi-shi, Chiba 274, Japan

(*Received* 14 *October* 1997)

Key Word Index—*Camellia sasanqua*; Theaceae; seed oil; *seco*-triterpene alcohol; sasanquol; antioedema; TPA-induced ear oedema.

Abstract—A novel 3,4-*seco*-triterpene alcohol, named sasanquol, was isolated from the non-saponifiable lipid of sasanqua oil from the seeds of *Camellia sasanqua*. Its structure was established to be 3,4-*seco*-D:B-friedobacchara-4,21-dien-3-ol by spectroscopic methods. This is the first example of naturally occurring triterpene with a D:B-friedobaccharane skeleton. The 50% inhibitory dose of this compound against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear inflammation (1 μg per ear) in mice was 0.4 mg per ear.

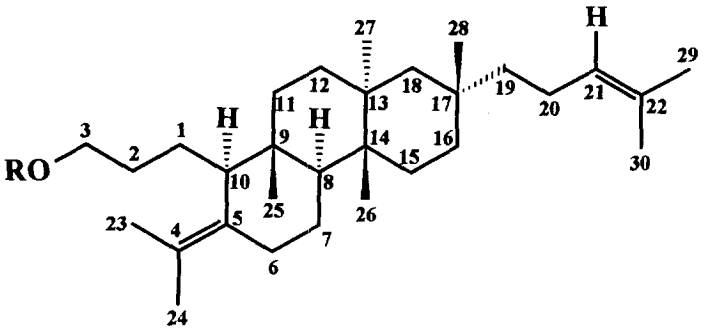
INTRODUCTION

The seeds of *Camellia sasanqua* Thunb. yield a non-drying oil which is similar in composition to camellia oil from the seed of *C. japonica* L. [1]. Sasanqua oil is occasionally used, for example as a hair oil and an ointment base, as a replacer of camellia oil [2]. Theacea seed oils including camellia and sasanqua oils possess as a characteristic feature significant amounts of euphane/tirucallane-type compounds, i.e., butyrospermol (eupha-7,24-dien-3β-ol) and Δ^7-tirucallol (tirucalla-7,24-dien-3β-ol), in the triterpene alcohol fractions [3–6]. Our recent investigation on the constituents of the triterpene alcohol fractions separated from the non-saponifiable lipids (NSL) of camellia and sasanqua oils led to the isolation and characterization of seven novel compounds along with 20 known compounds [7]. In this paper, we described the characterization and the anti-inflammatory effect of a further novel triterpene alcohol (**1**) named sasanquol, isolated from the NSL of sasanqua oil.

RESULTS AND DISCUSSION

Compound **1** was isolated as the acetyl derivative **2** from the acetylated triterpene alcohol fraction separated from the NSL of sasanqua oil. Its molecular formula was determined as $C_{32}H_{54}O_2$ on the basis of the HR mass spectrum (*m/z* 470.4092 $[M]^+$). Its IR spectrum indicated the presence of an acetoxyl group (1244, 1737 cm^{-1}) and a trisubstituted double bond (825 cm^{-1}). The ^1H NMR spectrum showed four olefinic methyl singlets (δ 1.60, 1.63, 1.65, 1.67), corresponding to two isopropylidene groups and indicating it to be a tricyclic *seco*-triterpene. The presence of the signals of an acetoxy methyl (δ 2.04) and an oxymethylene (δ 4.02, *t*) group suggested a 3,4-*seco*-triterpene-3-yl acetate structure [8, 9]. Further distinctive ^1H signals were those of four tertiary methyl singlets (δ 0.88, 0.96, 0.99, 1.07) which, along with the absence of a methyl doublet, suggested that the tricyclic-ring system was constituted with all six-membered rings. These data, in combination with the mass fragmentations observed at *m/z* 387 [loss of one C_6H_{11} (C-19–C-22, and C-29 and C-30) of the two side-chains], 287 [loss of both side-chains $C_5H_8O_2$ (CH_2=$CHCH_2OAc$) and C_6H_{11}], and 69 $[CH_2CH$=$C(Me)_2]^+$ (C-20–C-22, and C-29 and C-30; base peak), indicated either a baccharane- [10, 11], D:B-friedobaccharane- [12] or a lemmaphyllane (D:C-friedo- 18,19-*seco*-lupane)- [7, 10] skeletal structure with a 3,4-*seco*-triterpene-3-yl acetate moiety. The mass fragmentations and an olefinic methine signal (δ 5.08, *tt*) in the ^1H NMR spectrum suggested the presence of a C_6-side-chain containing an isopropylidene functionality [7, 10]. The other isopropylidene group was deduced to be located at C-4 [8, 9]. Further fragment ions observed at *m/z* 274

* Author to whom correspondence should be addressed.



(1) R = H
(2) R = Ac

($C_{20}H_{34}$) [8, 9, 10, 13], corresponding to the loss of *seco*-ring A and ring B be cleavages of the C-7–C-8 and C-9–C-10 bonds, and 163 ($C_{12}H_{19}$; 274 − C_6H_{11}) indicated the absence of a methyl group at C-10 and eliminated the possibility of the baccharane- and lemmaphyllane-skeletal structure. From the foregoing, compound **2** was assigned a 3,4-*seco*-D:B-friedobaccharа-4,21-dien-3-yl acetate structure. Analysis of the ^{13}C DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC spectra, and the ^{13}C and ^{1}H NMR spectral comparison of **2** with helianyl acetate [3,4-*seco*-19(10 → 9)*abeo*-8α,9β,10α-tirucalla-4,24-dien-3-yl acetate] (Table 1) [9]* and lemmaphylla-7,21-dien-3β-yl acetate (D:C-friedo-18,19-*seco*-lupa-7,19-dien-3β-yl acetate) [7] confirmed the above assumption.

The configuration of **2** was confirmed by difference NOE and NOESY spectroscopy. Compound **2** showed a significant NOE correlations between [H-26(14β-Me)–H-25(9β-Me)–H-23] and [H-27(13α-Me)–H-8–H-10] which were consistent with those observed for helianyl acetate [9], and between [H-26(14β-Me)–H-16β, H-18β–H-28(17β-Me)] which were consistent with those observed for lemmaphylla-7,21-dien-3β-yl acetate [7]. This suggested that **2** had a *trans-anti-trans* configuration in terms of the B/C/D-ring junctures orienting H-25, H-26 and H-28 to the β-face and H-8, H-10 and H-27 to the α-face of the ring-system and revealed the 3,4-*seco*-D:B-friedobaccharane skeletal structure for **2**. The most stable conformation of **2** with minimum steric energy was simulated using MacroModel and drawings [14, 15] (Fig. 1). This conformation of **2** was fairly consistent with the results from the NOE experiment carried out in solution. Alkaline hydrolysis of **2** yielded sasanquol (**1**; 3,4-*seco*-D:B-friedobaccharа-4,21-dien-3-ol; $C_{30}H_{52}O$; $[M]^+$, *m/z* 428.4024).

Sasanquol (**1**) is the first example of a naturally occurring triterpene possessing a D:B-friedobaccharane skeleton although several triterpenes with this skeleton have previously been synthesized, along with baccharane triterpenes, from a shionane triterpene by backbone rearrangement induced by boron trifluoride treatment [12]. D:B-Friedo baccharane triterpene has been postulated as a biosynthetic intermediate between baccharane triterpene and shionane triterpene in the biogenetic sequence leading to dammarane triterpene [16]. Sasanqua oil has been shown to contain bacchara-12,21-dien-3β-ol and dammaradienol (dammara-20,24-dien-3β-ol) as the triterpene alcohol constituents [7]. Although a number of 3,4-*seco*-triterpenes are known to occur in nature [17, 18], only a few compounds possessing a 3-hydroxyl group have so far been reported as natural products, i.e., 3,4-*seco*-dammara-4,24-dien-3-ol, isolated from *Abrotanella forsterioides* (Compositae) as the acetyl derivative [8] and from the pollen grains of *Helianthus annuus* (Compositae) [19], helianol from the flowers of *H. annuus* [9] and several other Compositae plants [20], and 3,4-*seco*-D: B-friedo-B′:A′-neogammacer-4(23)-en-3,5α-diol and its 5β-epimer from *Euphorbia supina* (Euphorbiaceae) [21].

Triterpene alcohols and plant sterols have recently been demonstrated to inhibit TPA-induced inflammation in mice [20, 22, 23]. Sasanquol (**1**) exhibited marked inhibitory activity against TPA-induced inflammation (1 μg per ear) and its 50% inhibitory dose was 0.4 mg per ear which was at a level almost corresponding to that of indomethacin (0.3 mg per ear), a commercially available anti-inflammatory drug, and was far more inhibitive than quercetin (3,3′,4′,5,7-pentahydroxyflavone) (1.6 mg per ear), a known inhibitor of TPA-induced inflammation in mice.

EXPERIMENTAL

Crystallizations from MeOH. Mp: uncorr.; Ag^+-TLC: silica gel-$AgNO_3$ (4:1, w/w) with cyclohexane–

* The assignment of a 20*R*-chirality (euphane-skeleton) for helianol [9] was erroneous, and we would like to revise the structure of the triterpene to a 20*S*-chirality (tirucallane-skeleton).

Table 1. ^{13}C and ^{1}H NMR spectral data for sasanquol (**1**) and sasanquyl acetate (**2**) ($CDCl_3$)

	1		**2**	
C	^{13}C	^{1}H	^{13}C	^{1}H
1	26.5	1.30, 1.55	26.6	1.29, 1.51
2	32.1	1.56 (2H)	27.8	1.58, 1.62
3	63.9	3.61 (*t*, 6.4)*	65.3	4.02 (*t*, 6.6)
4	122.1	—	122.2	—
5	134.5	—	134.3	—
6	23.7	2.32 (α), 1.94 (β)	23.9	2.32 (α), 1.94 (β)
7	21.9	1.46 (α), 1.40 (β)	21.9	1.46 (α), 1.37 (β)
8	42.9	1.37 (*dd*, 9.5, 13.9)	43.1	1.35
9	38.6	—	38.6	—
10	54.9	2.34	54.8	2.34
11	38.3	1.63 (α), 1.54 (β)	38.3	1.63 (α), 1.54 (β)
12	32.8	0.85 (α), 1.53 (β)	32.8	0.84 (α), 1.53 (β)
13	36.6	—	36.6	—
14	38.9	—	38.5	—
15	29.3	1.30 (α), 1.15 (β)	29.3	1.30 (α), 1.13 (β)
16	34.6	1.29 (α), 1.55 (β)	34.6	1.27 (α), 1.54 (β)
17	31.9	—	31.9	—
18	44.4	1.12 (α), 1.23 (β; *d*, 13.9)	44.4	1.12 (α), 1.23 (β; *d*, 14.7)
19	43.2	1.13, 1.67	43.2	1.14, 1.68
20	23.1	1.84, 1.98	23.2	1.84, 1.97
21	125.3	5.08 (*tt*, 1.5, 7.0)	125.3	5.08 (*tt*, 1.5, 7.0)
22	130.7	—	130.7	—
23	20.5†	1.64 (*s*)	20.7	1.63 (*s*)
24	20.8†	1.64 (*s*)	20.5	1.65 (*s*)
25	19.4	1.00 (*s*)	19.4	0.99 (*s*)
26	15.4	0.96 (*s*)	15.4	0.96 (*s*)
27	20.3	1.07 (*s*)	20.3	1.07 (*s*)
28	32.9	0.88 (*s*)	32.9	0.88 (*s*)
29	25.7	1.67 (*br s*)	25.7	1.67 (*br s*)
30	17.6	1.60 (*br s*)	17.6	1.60 (*br s*)
COMe	—	—	171.3	—
COMe	—	—	21.1	2.04 (*s*)

* Figures in parentheses denote *J* values (Hz). If not otherwise specified in parentheses, multiplicity of ^{1}H NMR signals was not determined.

† Assignment interchangeable.

EtOAc (9:1); HPLC: C_{18} silica columns [HPLC I: Superiorex ODS S 5 μm column, 25 cm × 10 mm i.d. (Shiseido Co., Ltd, Tokyo) temp. 25°; HPLC II: TSK ODS-120A 5 μm column, 25 cm × 10 mm i.d. (Toso Co., Tokyo), temp. 25°], MeOH as mobile phase (flow rate 4 ml min^{-1}); GC: DB-17 fused silica capillary column (30 m × 0.3 mm i.d., column temp. 275°). RR_t on HPLC and GC expressed relative to cholesteryl (cholest-5-en-3β-yl) acetate. EI-MS (70 eV): probe; ^{1}H NMR (400 MHz) and ^{13}C NMR (100.6 MHz): $CDCl_3$ with TMS (^{1}H NMR) and $CDCl_3$ at δ 77.0 (^{13}C NMR) as int. standard; IR: KBr. Acetylation: Ac_2O–pyridine, at room temp. overnight. Hydrolysis of acetate: 5% KOH in MeOH, at room temp. overnight. Source of crude sasanqua oil was described previously [7]. The NMR signal assignments for **1** and **2** were aided by ^{13}C DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC, NOESY and difference NOE spectroscopy.

Isolation procedure

Alkaline hydrolysis (5% KOH in MeOH, reflux, 3 hr) of crude sasanqua oil (1 kg) followed by diisopropyl ether extraction yielded neutral NSL (4.5 g). The NSL was chromatographed over a silica gel (250 g) column with hexane, hexane–EtOAc (9:1), hexane–EtOAc (4:1) as eluants. The hexane–EtOAc (9:1) eluted a fraction, which after rechromatography over silica gel, yielded a triterpene alcohol fraction (2.2 g) which, upon acetylation, gave an acetylated fraction (2.0 g). Crystallization of the acetate from Me_2CO–MeOH yielded β-amyrin (olean-12-en-3β-yl) acetate (0.35 g) as a solid mass and a filtrate (1.65 g). Ag^{+}-TLC of the filtrate afforded three major bands of which the second bulky band (R_f 0.50–0.70) from the solvent front yielded a fraction (0.85 g) which was composed mainly of the acetates of butyrospermol and Δ^{7}-tirucallol. HPLC of the fraction under the

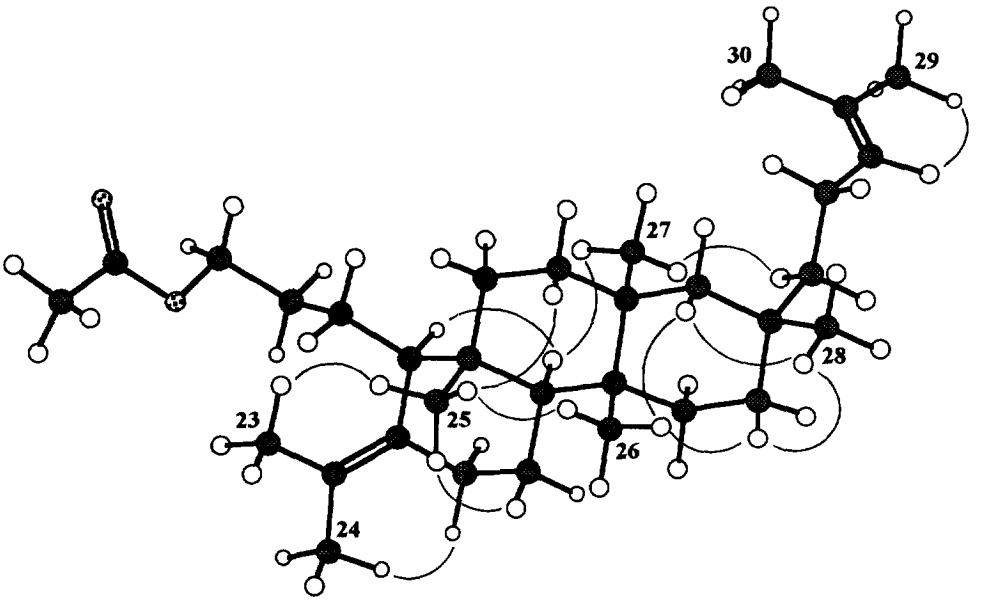


Fig. 1. Minimum energy conformation and some representative NOE correlations (—) for sasanquyl acetate (**2**).

condition I followed by II eventually yielded **2** (15 mg). Isolation and characterization of other triterpene alcohol constituents as the acetyl derivatives were described previously [7].

Sasanquyl acetate (**2**)

Mp 99–102° (fine needles). RR_t: 0.67 (HPLC I), 0.33 (HPLC II), 1.31 (GC). IR ν_{max} cm^{-1}: 1737, 1244, 825, 805; MS *m/z* (rel. int.): 470 $[M]^+$ (3), 455 (1), 387 (2), 287 (21), 274 (13), 259 (6), 249 (1), 245 (3), 231 (3), 223 (7), 205 (13), 203 (10), 191 (8), 189 (10), 163 (21), 69 (100); HR-MS *m/z*: 470.4092 ($C_{32}H_{54}O_2$, requires 470.4120), 387.3316 ($C_{26}H_{43}O_2$, requires 387.3261), 287.2705 ($C_{21}H_{35}$, requires 287.2736), 274.2682 ($C_{20}H_{34}$, requires 274.2659), 163.1455 ($C_{12}H_{19}$, requires 163.1485), 69.0702 (C_5H_9, requires 69.0703); ^{13}C NMR and ^1H NMR: Table 1. On alkaline hydrolysis, **2** yielded a free alcohol (**1**).

Sasanquol (**1**)

Mp 89–91° (fine needles). IR ν_{max} cm^{-1}: 3417, 825; MS *m/z* (rel. int.): 428 $[M]^+$ (11), 413 (3), 287 (27), 274 (18), 259 (8), 245 (3), 231 (4), 217 (4), 205 (14), 203 (8), 191 (7), 189 (9), 181 (10), 163 (17), 69 (100); HR-MS *m/z*: 428.4024 ($C_{30}H_{52}O$, requires 428.4016); ^{13}C NMR and ^1H NMR: Table 1.

Assay of TPA-induced inflammation in mice

The assay procedures were the same as those described in our previous article [20, 22, 23].

REFERENCES

1. In *The Lipid Handbook*, ed. F. D., Gunston, J. L. Harwood and F. B. Padley, 2nd edn. Chapman and Hall, London, 1994, p. 103.
2. In *Encyclopedia of Natural Medicine*, ed. T. Okuda. Hirokawa Publ. Co., Tokyo, 1986, p. 171.
3. Itoh, T., Tamura, T. and Matusmoto, T., *Lipids*, 1974, **9**, 173.
4. Itoh, T., Tamura, T. and Matusmoto, T., *Lipids*, 1976, **11**, 434.
5. Itoh, T., Uetsuki, T., Tamura, T. and Matsumoto, T., *Lipids*, 1980, **15**, 407.
6. Akihisa, T., Kokke, W. C. M. C. and Tamura, T., in *Physiology and Biochemistry of Sterols*, ed. G. W. Patterson and W. D. Nes. American Oil Chemists' Society, Champaign, Illinois, 1991, p. 172.
7. Akihisa, T., Yasukawa, K., Kimura, Y., Takase, S., Yamanouchi, S. and Tamura, T., *Chemical and Pharmaceutical Bulletin*, 1997, **45**, 2016.
8. Greger, H., Zdero, C. and Bohlmann, F., *Phytochemistry*, 1983, **22**, 2085.
9. Akihisa, T., Oinuma, H., Yasukawa, K., Kasahara, Y., Kimura, Y., Takase, S., Yamanouchi, S., Takido, M., Kumaki, K. and Tamura, T., *Chemical and Pharmaceutical Bulletin*, 1996, **44**, 1255.
10. Masuda, K., Shiojima, K. and Ageta, H., *Chemical and Pharmaceutical Bulletin*, 1983, **31**, 2530.
11. Akihisa, T., Kimura, Y. and Tamura, T., *Phytochemistry*, 1994, **37**, 1413.
12. Tachibana, K., Tori, M., Moriyama, Y., Tsuyuki, T. and Takahashi, T., *Bulletin of the Chemical Society of Japan*, 1977, **50**, 1552.

13. Gamalath, C. B., Gunatilaka, A. A. L. and Subramaniam, S. J., *Journal of the Chemical Society, Perkin Transactions* 1, 1989, 2259.
14. MacroModel Ver. 6.0 with extended MM3 parameters. The conformation with minimum steric energy was obtained through a Metropolis Monte-Carlo procedure. Drawings were performed by Chem3D program (Cambridge Scientific Computing Inc. Cambridge, MA, U.S.A.).
15. Mohamadi, F., Richards, N. G. L., Guida, W. C., Liskamp, R., Lipton, M., Caulfield, C., Chang, G., Hendrickson, T. and Still, W. C., *Journal of Computational Chemistry*, 1990, **11**, 440.
16. Shoji, N., Umeyama, A., Taira, Z., Takemoto, T., Nomoto, K., Mizukawa, K. and Ohizumi, Y., *Journal of the Chemical Society, Chemical Communications*, 1983, 871.
17. Baas, J. B., *Phytochemistry*, 1985, **24**, 1875.
18. In *Dictionary of Terpenoids*, Vol. 2, ed. J. D. Connolly and R. A. Hill. Chapman and Hall, London, 1991, p. 119.
19. Ohmoto, T., Udagawa, M. and Yamaguchi, K., *Shoyakugaku Zasshi*, 1986, **10**, 172.
20. Akihisa, T., Yasukawa, K., Oinuma, H., Kasahara, Y., Yamanouchi, S., Takido, M., Kumaki, K. and Tamura, T., *Phytochemistry*, 1996, **43**, 1255.
21. Tanaka, R., Matsunaga, S., Ishida, T. and Shingu, T., *Tetrahedron Letters*, 1989, **30**, 1661.
22. Yasukawa, K., Takido, M., Matsumoto, T., Takeuchi, T. and Nakagawa, S., *Oncology*, 1991, **48**, 72.
23. Yasukawa, K. and Akihisa, T., *Recent Research Developments in Oil Chemistry*, 1997, **1**, 115.