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24-METHYLPOLLINASTANONE, RELATED TRITERPENOIDS AND STEROLS FROM *COSTUS TONKINENSIS**

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Key Word Index—*Costus tonkinensis* Gagnep.; Zingiberaceae; 14α-methyl-9,19-cyclocholestane type triterpenoids; deuterium labelling; phytosterols; α-amyrin; β-amyrin.

Abstract—A series of scarce triterpenoid alcohols and ketones of the 14α-methyl-9,19-cyclocholestane type has been isolated from roots and aerial parts of *Costus tonkinensis*. 24-Methylpollinastanone identified by GC-mass spectrometry including deuterium labelling experiments was found as a natural product for the first time. In addition, α-amyrin, β-amyrin, several common phytosterols and some 7-oxosterols were identified.

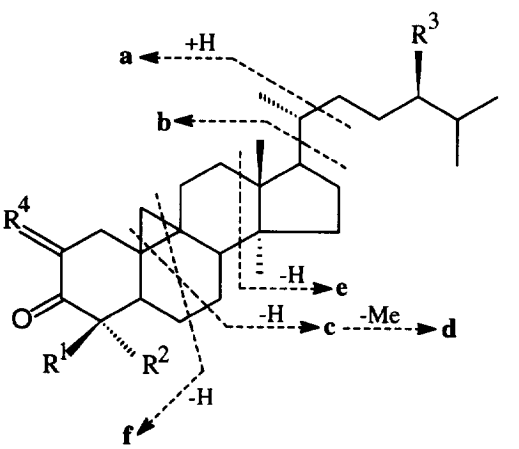
INTRODUCTION

Costus tonkinensis Gagnep. belonging to the Zingiberaceae family is an endemically occurring plant from South-East Asia with the main area located in the Tonking region of North Vietnam [1]. Its constituents have not been studied before. In continuation of our phytochemical studies on plants of Vietnam [2] the present paper deals with the isolation and identification of triterpenoid and steroidal constituents from *C. tonkinensis*.

RESULTS AND DISCUSSION

Several triterpenes with a 14α-methyl-9,19-cyclocholestane skeleton were isolated from the *n*-hexane extract of roots and aerial parts of *Costus tonkinensis*. The scarce triterpene ketones 29-norcycloartanone (**1**), cycloartanone (**2**), cycloeucalanone (**3**), 24-methylenecycloartanone (**4**) and 24-methylpollinastanone (**5**) were identified by GC-mass spectrometry in comparison with data from the literature. Furthermore, α-amyrone and β-amyrone were found in the ketone fraction. To obtain more detailed information concerning the degree of methylation at C-4, as well as the assignment of important key ions in the EI mass spectra, the triterpene ketone fraction was deuterated in the α-position (C-4) by base catalyzed exchange with deuterated water.

The mass spectral fragmentation of the triterpene ketones **1–5** is mainly characterized by cleavages of the side chain (ions of type **a** and **b**) as well as in ring B (ions **c** and **d**) [3–5] (Scheme 1, Table 1). The key ion of type **e** resulting from a ring C cleavage has not been discussed in the literature before. The **f**-type ion at *m/z* 136 appears only in the 4α-methyl compounds **1** and **3**. As indicated by a corresponding mass shift towards *m/z* 138 in the deuterated derivatives **1d** and **3d**, respectively, its origin is accompanied by a hydrogen transfer from C-2 or C-4 of the charged species. Generally, the mass spectra of the α-deuterated derivatives **1d–5d** are in agreement with the discussed fragment ions. Using the key ions **a–f** it is possible to determine not only the triterpene skeleton but also the



Scheme 1. Mass spectral fragmentation of the 3-keto-triterpenes **1–5**.

* Dedicated to Professor Klaus Schreiber on the occasion of his 70th birthday.

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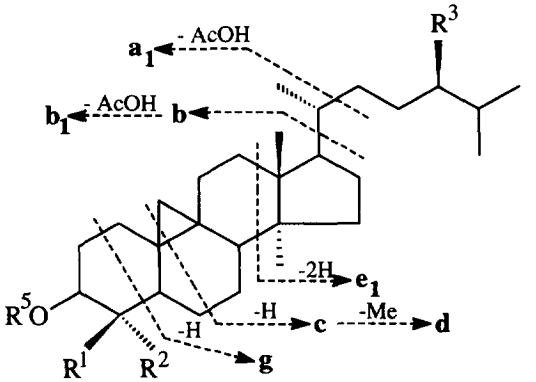
Table 1. Key ions in the EI-mass spectra of the triterpenoid ketones **1–5** [*m/z* (rel. int.)]*

Compound	R^1	R^2	R^3	R^4	M^+	$[M-CH_3]^+$	a	b	c	d	e	f
1	H	CH_3	H	H_2	412(10)	397(7)	328(7)	299(25)	288(4)	273(3)	207(8)	136(22)
1d	D	CH_3	H	D_2	415(14)	400(8)	331(7)	302(26)	288(7)	273(6)	207(11)	138(22)
2	CH_3	CH_3	H	H_2	426(7)	411(7)	342(4)	313(25)	288(9)	273(3)	207(5)	—
2d	CH_3	CH_3	H	D_2	428(13)	413(10)	344(5)	315(25)	288(17)	273(13)	207(9)	—
3	H	CH_3	CH_3	H_2	426(13)	411(8)	328(9)	299(40)	302(3)	287(2)	221(5)	136(25)
3d	D	CH_3	CH_3	D_2	429(20)	414(11)	331(11)	302(44)	302(44)	287(5)	221(8)	138(24)
4	CH_3	CH_3	$=CH_2$	H_2	438(7)	423(5)	340(5)†	313(8)	300(5)	285(3)	219(5)	—
4d	CH_3	CH_3	$=CH_2$	D_2	440(10)	425(7)	342(6)†	315(7)	300(7)	285(4)	219(9)	—
5	H	H	CH_3	H_2	412(7)	397(5)	314(5)	285(34)	302(1)	287(2)	221(4)	—
5d	D	D	CH_3	D_2	416(7)	401(3)	318(4)	289(24)	302(2)	287(8)	221(5)	—

* The base peak (100%) of all triterpenoid ketones was at *m/z* 95.
† (-2H).



Scheme 2. Mass spectral fragmentation of the triterpene alcohols **7–13** (as acetates).

degree of methylation at carbons C-4, C-14 and in the side chain (C-24).

Triterpene ketones with the 14α-methyl-9,19-cyclocholestane skeleton are scarce compounds in plants compared with the corresponding alcohols. 24-Methylenecycloartanone (**4**) has been found in several *Tillandsia* species (Bromeliaceae) [6, 7], *Euphorbia piscatoria* (Euphorbiaceae) [8] and *Melia azedarach* (Meliaceae) [9]. Cycloeucalanone (**3**) was also isolated from *Musa sapientum* (Musaceae) [10]. 29-Norcycloartanone (**1**) was hitherto only detected in roots of *Costus speciosus* [11]. To the best of our knowledge a natural occurrence of 24-methylpollinastanone (**5**) has not been described before. However, it was detected as a metabolite of several 4α-methyl and 4,4-dimethyl-9,19-cyclocholestane type triterpenes after application of membrane-bound oxidative enzymatic systems [12]. The triterpene ketones **1–5** along with α-amyrone and β-amyrone were only found in the roots of *C. tonkinensis*, but not in the aerial parts.

In addition to the triterpene ketones **1–5** the corresponding alcohols with a 14α-methyl-9,19-cyclocholestane skeleton, 29-norcycloartanol (**6**), cycloartanol (**7**), cycloeucalenol (**8**), cycloeucalanol (**9**), 24-methylenecycloartanol (**10**), pollinastanol (**11**), 24-methylenepollinastanol (**12**) and 24-methylpollinastanol (**13**) were identified from *C. tonkinensis* by GC-mass spectrometry of their acetates (Table 2).

The fragmentation behaviour of the triterpene alcohols **7–13** is similar to that of the ketones **1–5**. However, in contrast to the ketones the alcohol acetates display a cleavage through ring A (ion **g**) but no **f**-type ion (Scheme 2). Pollinastanol (**11**) was also found in *Costus spiralis*, a South American genus [13]. 24-Methylenepollinastanol (**12**) and 24-methylpollinastanol (**13**) were detected for the first time in *Astasia longa* (Euglenaceae) [14].

Table 3 summarizes the occurrence of the triterpenoids and phytosterols in *Costus tonkinensis*. In addition to the triterpene alcohols with the 14α-methyl-9,19-cyclocholestane skeleton (**6–13**) traces of α-amyrin and β-amyrin were identified.

The phytosterols from roots were analyzed as their acetates by GC-mass spectrometry in comparison with authentic samples [15], and shown to be a mixture of cholesterol (7%), brassicasterol (2%), campesterol (13%), stigmasterol (25%), sitosterol (49%) and sitostanol (4%). The aerial parts contained a similar phytosterol mixture with respect to their pattern and their relative composition. However, the 7-oxosterols (7-oxocholesterol, 7-oxocampesterol, 7-oxostigmasterol and 7-oxositosterol), which were identified by comparison with authentic samples [16], were found only in the aerial parts (Table 3).

EXPERIMENTAL

MPLC. L 6250 Preparative Intelligent Pump (Merck/Hitachi); Latek glass column M2-48, i.d. 20 mm, length 48 cm, pressure 30 mbar; silica gel 60 PF_{254} (5–40 μm).

GC-MS. MD 800 (Fisons Instruments), EI (70 eV); column DB-5MS (15 m × 0.32 mm, 0.25 μm film thickness); source temp. 200°; injection temp. 250°, interface temp. 300°, carrier gas He, flow rate: 1.3 ml min^{-1}; splitless injection; temp. program: 1 min at 170°, then elevated to 270° with 25° min^{-1}, then raised to 290° with 2° min^{-1}. The relative retention times (RR_t) were calculated with respect to 5α-cholestane (R_t 5.91 min).

Plant material. C. tonkinensis was collected in

Table 2. Key ions in the EI-mass spectra of the acetates of the triterpenoid alcohols **6**–**13** [*m/z* (rel. int.)]

Compound	R^1	R^2	R^3	R^5	M^+	$[M-CH_3]^+$	$[M-HOAc]^+$	$[M-HOAc-CH_3]^+$	$\mathbf{a}_1$	**b**	$\mathbf{b}_1$	**c**	**d**	$\mathbf{e}_1$	**g**	Base peak
6	H	CH_3	H	Ac	456 (4)	441 (5)	396 (47)	381 (70)	311 (3)	343 (5)	283 (33)	288 (16)	273 (10)	206 (13)	341 (15)	55 (100)
7	CH_3	CH_3	H	Ac	470 (3)	455 (5)	410 (29)	395 (40)	325 (3)	357 (5)	297 (26)	288 (28)	273 (13)	206 (7)	341 (15)	95 (100)
8	H	CH_3	$=CH_2$	Ac	468 (2)	453 (4)	408 (44)	393 (43)	311 (3)/ 309 (4)*	343 (1)/ 341 (1)*	283 (10)/ 281 (11)*	300 (5)	285 (5)	—	353 (4)	55 (100)
9	H	CH_3	CH_3	Ac	470 (3)	455 (3)	410 (36)	395 (51)	311 (3)	343 (4)	283 (24)	302 (9)	287 (5)	220 (7)	355 (9)	55 (100)
10	CH_3	CH_3	$=CH_2$	Ac	482 (3)	467 (3)	422 (35)	407 (27)	325 (1)	357 (1)/ 355 (1)*	297 (9)/ 295 (5)*	300 (9)	285 (6)	—	353 (3)	55 (100)
11	H	H	H	Ac	442 (5)	427 (7)	382 (62)	367 (81)	297 (5)	329 (7)	269 (57)	288 (7)	273 (7)	206 (27)	341 (7)	95 (100)
12	H	H	$=CH_2$	Ac	454 (3)	439 (5)	394 (51)	379 (52)	297 (6)/ 295 (9)*	329 (4)/ 327 (3)*	269 (27)/ 267 (21)*	300 (3)	285 (3)	–	—	69 (100)
13	H	H	CH_3	Ac	456 (5)	441 (5)	396 (56)	381 (75)	297 (6)	329 (9)	269 (55)	302 (7)	287 (5)	220 (19)	355 (5)	95 (100)

* (-2H).

Table 3. Triterpenoids and phytosterols in *C. tonkinensis*

Triterpene	RR_t*	Roots	Aerial parts
29-Norcycloartanone (**1**)	1.41	+	–
β-Amyrone	1.47	+	–
Cycloartanone (**2**)	1.49	+	–
α-Amyrone	1.54	+	–
Cycleucalanone (**3**)	1.57	+	–
24-Methylenecycloartanone (**4**)	1.64	+	–
24-Methylpollinastanone (**5**)	1.49	+	–
29-Norcycloartanol (**6**)	1.60†	+	–
β-Amyrin	1.68†	(+)	(+)
Cycloartanol (**7**)	1.73†	+	+
Cycloeucalenol (**8**)	1.77†	+	+
Cycloeucalanol (**9**)	1.78†	+	+
α-Amyrin	1.79†	(+)	(+)
24-Methylenecycloartanol (**10**)	1.90†	+	+
Pollinastanol (**11**)	1.51†	+	–
24-Methylenepollinastanol (**12**)	1.68†	(+)	–
24-Methylpollinastanol (**13**)	1.70†	+	–
Cholesterol	1.40†	+	+
Brassicasterol	1.46†	(+)	(+)
Campesterol	1.56†	+	+
Stigmasterol	1.61†	+	+
Sitosterol	1.72†	+	+
Sitostanol	1.74†	+	+
7-Oxocholesterol	1.67	–	+
7-Oxocampesterol	1.87	–	+
7-Oxostigmasterol	1.93	–	+
7-Oxositosterol	2.06	–	+

* RR_t = relative retention time with respect to 5*α*-cholestane; † acetates; (+) traces.

March 1995 in the National Park Cuc Phuong (North Vietnam) and identified by Dr Tran Dinh Dai. A voucher specimen of *C. tonkinensis* (no. 521 HN) is deposited at the Institute of Ecology and Natural Resources of Vietnam, Nghia Do, Tu Liem, Hanoi.

Extraction and isolation. The air-dried roots (369 g) and aerial parts (417 g) were extracted 4 × with 95% MeOH, dried *in vacuo* and 50% of the methanolic extract (roots 11.7 g, aerial parts (15.5 g) was then partitioned with *n*-hexane.

Root material. A portion (1.6 g) of the *n*-hexane extract (1.73 g) was chromatographed on a silica gel column (80 g, Merck 60, 0.063–0.2 mm). Stepwise elution with an *n*-hexane–EtOAc gradient system (10:0, 9:1, 7:3, 5:5, 3:7, 0:10) was carried out with 650 ml of each gradient. Upon TLC monitoring ($CHCl_3$–MeOH, 95:5) three main frs (**FI**, **FII**, **FIII**) were obtained. Fr. **FI** (50 mg) eluted with *n*-hexane was further purified on a silica gel column (3 g, Merck 60, 0.04–0.063 mm) using $CHCl_3$–*n*-hexane (1:2, 1:1, 1:0) as eluents (2 ml frs, 25 ml of each gradient). In frs 14–20 (4 mg) 29-norcycloartanone (**1**), cycloartanone (**2**) cycloeucalanone (**3**), 24-methylenecycloartanone (**4**), *α*-amyrone and *β*-amyrone as

well as in frs 22–27 (11 mg) 24-methylpollinastanone (**5**) were identified by GC-MS (Table 1).

A portion (1 mg) of each of the ketone frs was deuterated by base-catalyzed exchange [17]. The following incorporations of deuterium were determined from the molecular ions:

Compound	D_0(%)	D_1(%)	D_2(%)	D_3(%)	D_4(%)
1d	2	11	35	52	—
2d	5	36	57	2	—
3d	1	6	36	57	—
4d	8	34	55	3	—
5d	2	1	11	39	47

Fr. FII (47 mg) was further purified on a silica gel column with $CHCl_3$ as eluent to give a triterpene alcohol fr. (33 mg). Upon acetylation of 2 mg with Ac_2O–pyridine at room temp. for 12 hr and subsequent GC-MS examination, 29-norcylcoartanol (**6**), cycloartanol (**7**), cycloeucalenol (**8**), cycloeucalanol (**9**), 24-methylenecycloartanol (**10**), α-amyrin and β-amyrin were identified (Tables 2 and 3).

The phytosterol containing fr. FIII (26 mg) was purified by prep. TLC (Merck 60 F_{254}, 1 mm) with $CHCl_3$–MeOH (95:5) as eluent. A portion (2 mg) of the purified fr (19 mg) was acetylated and examined by GC-MS. In addition to cholesterol, campesterol, stigmasterol, sitosterol, brassicasterol and sitostanol, the triterpene alcohols pollinastanol (**11**), 24-methylenepollinastanol (**12**) and 24-methylpollinastanol (**13**) were identified.

Aerial parts. A portion (6 g) of the *n*-hexane extract (13.56 g) was chromatographed stepwise on a silica gel column (400 g) eluting 20 ml fr with $CHCl_3$. The frs were monitored by TLC ($CHCl_3$–MeOH, 95:5). The triterpene alcohol containing fr. (20 mg) was further purified by MPLC with an *n*-hexane–EtOAc gradient system. The triterpene alcohol fraction (9 mg) obtained was recrystallized from MeOH; 2 mg were then acetylated and examined by GC-MS.

The phytosterol containing fr. (28 mg) was further purified by prep. TLC (Merck 60 F_{254}, 1 mm) with $CHCl_3$–MeOH (95:5) as eluent. The phytosterol mixture (4 mg) was acetylated and examined by GC-MS.

The 7-oxosterol containing fr. (265 mg) was chromatographed on a silica gel column (14 g) using *n*-hexane–EtOAc (7:3) as eluent. 29 mg of a mixt. consisting of 7-oxocholesterol, 7-oxocampesterol, 7-oxostigmasterol and 7-oxositosterol could be isolated and identified by GC-MS.

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