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TWO PHENOLIC *FRIEDO*-23,24-DINOROLEANANE TRITERPENES FROM *KOKOONA ZEYLANICA**

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Key Word Index—*Kokoona zeylanica*; Celastraceae; phenolic D:A-*friedo*-23,24-dinoroleananes; 23-*nor*-6-oxopristimerol; 23-*nor*-6-oxodemethylpristimerol; demethylzeylasteral; structure elucidation; biosynthesis.

Abstract—Two novel phenolic *dinor*-triterpenes isolated from *Kokoona zeylanica* have been identified as 23-*nor*-6-oxopristimerol and 23-*nor*-6-oxodemethylpristimerol on the basis of spectroscopic evidence and chemical interconversions. The biosynthetic significance of the co-occurrence of phenolic D:A-*friedo*-23, 24-dinoroleananes with phenolic and quinone-methide triterpenes in *K. zeylanica* is discussed.

INTRODUCTION

The phenolic-D:A-*friedo-nor*-oleananes form a unique group of triterpenoids represented by zeylasterone (**4**), which was first encountered in *Kokoona zeylanica* [2]. Subsequently four additional phenolic triterpenoids, zeylasteral (**5**), demethylzeylasterone (**6**), demethylzeylasteral (**7**) and 23-oxoisopristimein III (**9**) along with the quinone-methides pristimerin (**10**) and celastrol (**11**) were isolated from *K. zeylanica* and kokum soap [3–5]. A recent study [6] has revealed the presence of some of these phenolic triterpenoids in *K. reflexa* and *Celastrus paniculatus* but not in *Cassine balae* (*Elaeodendron balae*), *Gymnosporia emarginata* and *Pleurostylia opposita*, all belonging to the family Celastraceae. We now report on the isolation from *K. zeylanica* of the first two natural phenolic D:A-*friedo*-23, 24-dinoroleanane triterpenoids, 23-*nor*-6-oxopristimerol (**1**) and 23-*nor*-6-oxodemethylpristimerol (**2**).

RESULTS AND DISCUSSION

The ethyl acetate extract of the root outer bark of *K. zeylanica* afforded three yellow compounds. One of these was identified as demethylzeylasteral (**7**) [3]. The remaining two were new natural products and their structures were elucidated as 23-*nor*-6-oxopristimerol (**1**) and 23-*nor*-6-oxodemethylpristimerol (**2**).

23-*nor*-6-Oxopristimerol gave a positive response to both the Liebermann–Burchard test and the neutral ferric chloride test indicating it to be a phenolic triterpene [2]. IR bands at 3500–3000, 1720 and 1630 cm^{-1} indicated the presence of hydroxy groups, a saturated ester carbonyl group and an α,β-unsaturated ketone group, respectively. The ^1H NMR spectrum was similar to that of zeylasterone (**4**) except that an additional 1H singlet was present in the aromatic region at δ 7.80 which was attributed to H-4 (Table 1). It had comparable chemical shift values to those of *peri*-protons of related systems (e.g. xanthones, flavonoids etc.) [7]. The ^1H NMR spectrum of **1** also indicated the presence of five methyl groups attached to quaternary centres and a carbomethoxy group. In its MS, 23-*nor*-6-oxopristimerol (**1**) showed a significant peak at *m/z* 204 probably due to the trihydroxynaphthalene ion (**12**) formed as a result of a retro-Diels–Alder fragmentation of ring C (Scheme 1).

Methylation of 23-*nor*-6-oxopristimerol with excess diazomethane in ether afforded the dimethyl derivative **3**, $C_{31}H_{42}O_5$. The IR spectrum of this compound contained no absorptions due to hydroxy groups and the ^1H NMR spectrum was almost superimposable on that of **1** except that two additional 3H singlets were present in the methoxy region at δ 3.94 and 3.96 [cf. ^1H NMR data of trimethylzeylasterone (**8**)] (Table 1). As for the parent triterpene, the MS of **3** showed a significant peak at *m/z* 232 corresponding probably to the ion **13** (Scheme 1). The ^{13}C NMR spectrum of **3** (Table 2) provided additional evidence for the proposed structure. The signals were assigned with the help of DEPT spectra and by comparison with ^{13}C NMR shifts of zeylasterone (**4**), whose assignments have recently been revised from that reported previously [2–5] using ^1H–^{13}C shift correlated spectra [C. B. Gamlath, A. A. L. Gunatilaka, T. Tezuka and T. Kikuchi, unpublished results].

The more polar phenolic triterpene gave positive responses to both the Liebermann–Burchard test and the neutral ferric chloride test for phenolic triterpenes [2]. The MS with $[M]^+$ at *m/z* 452 (14 amu less than the $[M]^+$ of **1**) and the ^1H NMR spectrum which was almost superimposable on that of **1** except for the absence of the signal due to 20α-CO_2 Me (Table 1) indicated it to be the demethyl derivative of **1**, namely 23-*nor*-6-oxodemethylpristimerol (**2**). Methylation of **2** gave its trimethyl derivative which was identical with dimethyl 23-*nor*-6-oxopristimerol (**3**) obtained above.

It was possible that 23-*nor*-6-oxopristimerol (**1**) and 23-*nor*-6-oxodemethylpristimerol (**2**) were artefacts formed as a result of decarboxylation of the enolized β-keto acids

* Part 14 in the series Studies on Terpenoids and Steroids; For Part 13, see ref. [1].

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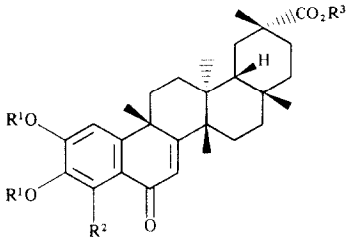
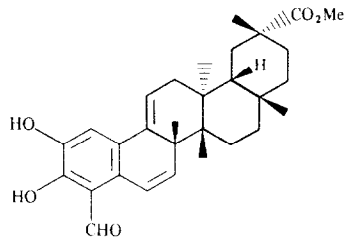
Table 1. ^{1}H NMR spectral data of some phenolic triterpenoids (60 MHz, $CDCl_3$, TMS as int. standard)

Compound	H-1	H-4	H-7	Me-9	Me-13	Me-14	Me-17	Me-20β	CO_2Me-4	CHO-4	CO_2Me-20α	$(OMe)_2$-2,3
1	7.00	7.80	6.36	1.53	1.28	0.53	1.10	1.20	—	—	3.53	—
2	6.91	7.43	6.28	1.50	1.28	0.63	1.11	1.21	—	—	—	—
3*	6.89	7.55	6.36	1.58	1.32	0.58	1.11	1.17			3.53	3.94, 3.96
4	7.35	—	6.48	1.35	1.34	0.54	1.12	1.18	—	—	3.54	—
7	7.23	—	6.33	1.56	1.31	0.73	1.11	1.20	—	11.0	3.53	—
8	6.95	—	6.22	1.60	1.32	0.60	1.11	1.17	3.93	—	3.53	3.82, 3.93

* Recorded at 500 MHz.



1 $R^1 = R^2 = H$; $R^3 = Me$
2 $R^1 = R^2 = R^3 = H$
3 $R^1 = R^3 = Me$; $R^2 = H$
4 $R^1 = H$; $R^2 = CO_2H$, $R^3 = Me$
5 $R^1 = H$; $R^2 = CHO$; $R^3 = Me$
6 $R^1 = R^3 = H$; $R^2 = CO_2H$
7 $R^1 = R^3 = H$; $R^2 = CHO$
8 $R^1 = R^3 = Me$; $R^2 = CO_2Me$



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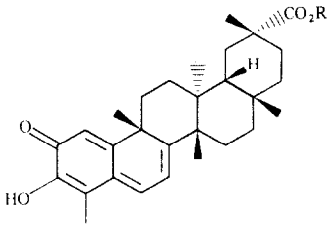


10 R = Me
11 R = H

4 and **6**, respectively, during extraction with hot ethyl acetate which can be slightly acidic. However, this possibility was ruled out as treatment of **4** with hot ethyl acetate even for a prolonged period did not afford even a trace amount of **1** (TLC control).

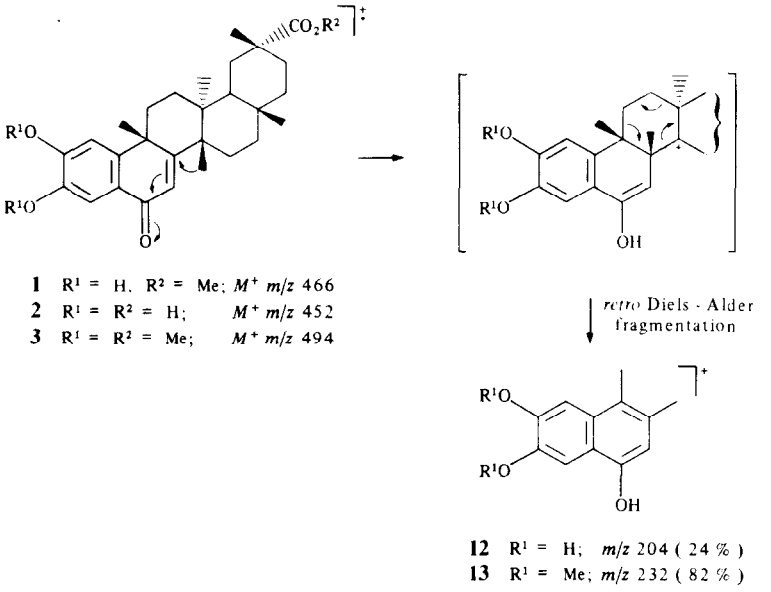
This constitutes the first report of the natural occurrence of phenolic D:A-*friedo*-23, 24-dinoroleanane triterpenes and is significant as these co-occur with quinone-methide and phenolic triterpenoids in *K. zeylanica*. Four pathways have been postulated for the biosynthetic origin of quinone-methide triterpenoids and their biosynthetic relationship with phenolic triterpenoids [2, 8-10]. Oxidative decarboxylation of the corresponding quinone-methide triterpenoids (**10** and **11**) or phenolic triterpenoids (**4** and **6**) would yield 23-*nor*-6-oxopristimerol (**1**) and 23-*nor*-6-oxodemethylpristimerol (**2**) as depicted in Scheme 2.

EXPERIMENTAL

General. The general experimental details were the same as those described previously [2]. IR: KBr; Optical rotations: 27°, $CHCl_3$; ^{1}H NMR: $CDCl_3$, 60 MHz unless otherwise stated, TMS as int. ref. ^{13}C NMR: 75 MHz. Multiplicity of carbon signals established by using DEPT measurements. Petrol refers to the fraction bp 60–80°.

Extraction of K. zeylanica *outer root bark.* The dried and powdered root outer bark (0.5 kg) of *K. zeylanica* collected at Kanneliya rain forest, Sri Lanka was successively and exhaustively extracted with hot petrol, C_6H_6 and EtOAc. Investigation of the hot petrol and C_6H_6 extracts have been reported previously [5]. The hot EtOAc extract on evapn afforded a black semisolid (4.7 g, 0.94%).

Isolation of 23-nor-6-*oxopristimerol* (**1**). The EtOAc extract (4.0 g) was chromatographed over a column of silica gel (mesh 70–230) made up in $CHCl_3$ and eluted with $CHCl_3$ containing



1 R^1 = H, R^2 = Me; M^+ *m/z* 466
2 R^1 = R^2 = H; M^+ *m/z* 452
3 R^1 = R^2 = Me; M^+ *m/z* 494

12 R^1 = H; *m/z* 204 (24 %)
13 R^1 = Me; *m/z* 232 (82 %)

Scheme 1. Mass spectral fragmentation of triterpenoids **1–3**.

Table 2. ^{13}C NMR spectral data of dimethyl 23-*nor*-6-oxopristimerol (**3**) and zeylasterone (**4**) (75 MHz, $CDCl_3$, TMS as int. standard)

C	3	4*	C	3	4*	C	3	4*
1	106.8†	113.8	11	33.8	34.4	21	29.8	29.84
2	147.8	152.9‡	12	29.8	29.78	22	34.8	34.9
3	150.0	153.5‡	13	39.5	39.7	23	—	173.8
4	106.7†	111.4	14	45.2	45.6	25	36.5	36.8
5	123.6	119.5	15	28.9	28.6	26	20.5	20.2
6	184.7	188.1	16	36.4	36.2	27	18.2	18.3
7	124.4	124.4	17	30.5	30.6	28	31.6	31.6
8	174.6	179.8	18	44.4	44.3	29	178.8	178.7
9	40.5	42.9	19	31.0	30.9	30	32.6	32.7
10	153.2	155.6	20	40.0	40.5	CO_2Me	51.6	51.6
						$(OMe)_2$	56.0	—

*Assignments are based on ^1H–^{13}C shift correlated spectra (C. B. Gamlath, A.A.L. Gunatilaka, T. Tezuka and T. Kikuchi, unpublished results).

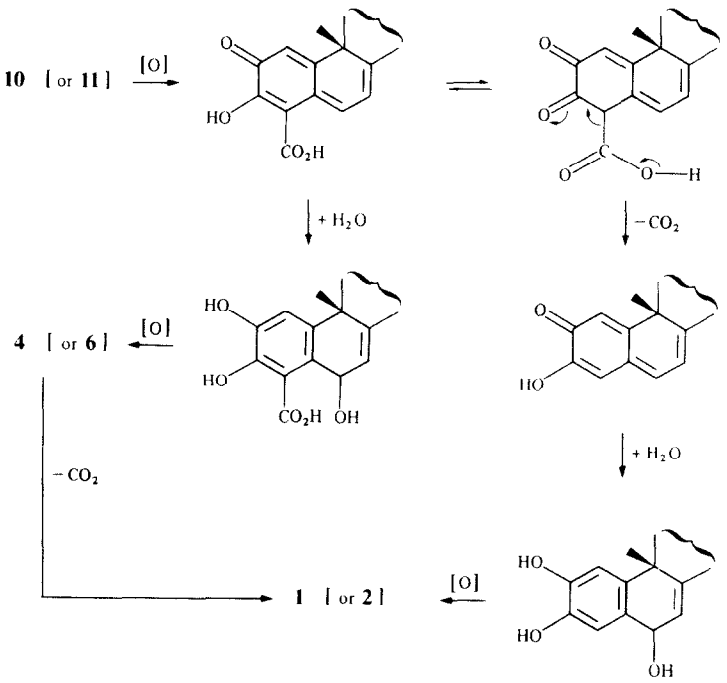
†‡ May be interchanged in any column.

increasing amounts of MeOH. The fraction eluted with 1% MeOH in $CHCl_3$ was further purified by prep. TLC (silica gel, MeOH–$CHCl_3$–C_6H_6 2:96:2) to yield 23-*nor*-6-oxopristimerol as a pale yellow solid (45 mg, 0.009%), mp 135–138°, $(\alpha)_D^{27}$ -74.9° IR γ_{max} cm^{-1} 3500–3000, 2925, 1720, 1630, 1570, 1510, 1455, 1370, 1290, 1210, 875 and 750; ^1H NMR: see Table 1; MS (rel. int.) *m/z*: 466 $[M]^+$ (45), ($C_{29}H_{38}O_5$), 451 (18) ($C_{28}H_{35}O_5$), 271 (13) ($C_{17}H_{19}O_3$), 243 (14) ($C_{15}H_{15}O_3$), 217 (23) ($C_{13}H_{13}O_3$), 204 (100) ($C_{12}H_{12}O_3$) and 189 (12) ($C_{11}H_9O_3$); $[M]^+$ 466.27515. $C_{29}H_{35}O_5$ requires 466.27192.

Isolation of demethylzeylasteral (**7**). The fraction eluted with 3% MeOH in $CHCl_3$ on further purification by prep. TLC yielded demethylzeylasteral (63 mg, 0.012%) identical with an authentic sample [5]. See Table 1 for ^1H NMR data.

Isolation of 23-*nor*-6-*oxodemethylpristimerol* (**2**). The fraction eluted with 5% MeOH in $CHCl_3$ on further purification by prep. TLC (silica gel, MeOH–$CHCl_3$–C_6H_6 1:8:1) afforded 23-*nor*-6-oxodemethylpristimerol as a yellow solid (32 mg, 0.0064%), mp 228–230°, $(\alpha)_D^{27}$ −102.0° UV λ_{max}^{EtOH} nm (log ε): 203 (4.29), 226 (3.96), 254 (4.05), 298 (3.70) and 335 (3.71); IR ν_{max} cm^{-1} 3500–3050, 2900, 1710, 1620, 1570, 1500, 1455, 1370, 1290 and 895; ^1H and ^{13}C NMR: see Tables 1 and 2, respectively; MS *m/z* (rel. int.) 452 $[M]^+$ (4), 437(1), 271(2), 243(4), 204(24), 149(8) and 83(100).

Methylation of 23-nor-6-*oxopristimerol*. 23-*nor*-6-Oxopristimerol (**1**) (17.5 mg) dissolved in Et_2O (2.5 ml) was treated with excess ethereal CH_2N_2 overnight. Removal of solvent and subsequent purification of the product by prep. TLC (silica gel, $CHCl_3$–MeOH 19:1) afforded dimethyl-23-*nor*-6-oxopristimerol (**3**) as a pale yellow semi-solid (17 mg) which resisted crystallization; IR ν_{max} cm^{-1} 2900, 1720, 1640, 1590, 1500, 1450, 1410, 1360, 1290, 1250, 1210, 1145, 1050, and 860; ^1H



Scheme 2. Possible biosynthetic relationship of phenolic and quinone-methide triterpenoids of *K. zeylanica*.

and ^{13}C NMR: see Tables 1 and 2, respectively; MS *m/z* (rel. int.) 494 $[M]^+$ (74) ($C_{31}H_{42}O_5$), 479 $[M-Me]^+$ (52) ($C_{30}H_{39}O_5$), 297 (10) ($C_{19}H_{21}O_3$), 272 (16) ($C_{17}H_{20}O_3$), 245 (23) ($C_{15}H_{17}O_3$), 232 (82) ($C_{14}H_{16}O_3$), 141 (54) ($C_8H_{13}O_2$); $[M]^+$ 494.30712. $C_{31}H_{42}O_5$ requires 494.30699.

Methylation of 23-nor-6-*oxodemethylpristimerol*. 23-*nor*-6-Oxodemethylpristimerol (25 mg) was methylated as above to yield dimethyl-23-*nor*-6-oxopristimerol (**3**) (24 mg), identical (co-TLC, Co-IR and ^1H NMR) with the above obtained sample.

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