Oumarone, Bissaone, and Aissatone, Unusual Prenylated Polyketides from *Harrisonia abyssinica*

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Fractionation of the *n*-hexane extract of the leaves of *Harrisonia abyssinica*, collected in Guinea, afforded three novel and unusual prenylated polyketides, which were named oumarone (**1**), bissaone (**2**), and aissatone (**3**). Their structures were elucidated by spectroscopic methods, mainly 1D and 2D NMR spectroscopy.

Harrisonia abyssinica Oliv. (Simaroubaceae) is a small tree or shrub used widely in African traditional medicine. Extracts of the bark and the root of this plant have been shown to exhibit in vitro antiviral, antibacterial, antifungal, and molluscicidal activities.[1] Chemical investigations have led to the identification of various steroids, limonoids, and chromones.[2] In this paper we report the isolation and structure elucidation of three novel and unusual prenylated polyketides, which were named oumarone (**1**), bissaone (**2**), and aissatone (**3**).

Compounds **1**−**3** were obtained from the *n*-hexane extract of powdered stem bark (1 kg) of *H. abyssinica*, collected in Sérédou, Guinea. The EIMS of compound **1** showed a molecular ion at *m*/*z* 236, while HREIMS indicated a molecular formula of $C_{15}H_{24}O_2$ (*m*/*z* 236.177). ^{13}C NMR signals at δ 196.38, 191.75, and 100.00 were in agreement with a β-diketone in its enolic form (**1a**). A series of five ^{13}C NMR signals with approximately double intensity could be assigned to two equivalent isoprenyl groups. The remaining CH and CH_3 groups (δ 49.05 and 25.15 in ^{13}C NMR) were correlated in a HSQC experiment with ^1H NMR signals at δ 2.17 (m) and δ 2.05 (s), respectively. $^1H-^1H$ COSY and $^1H-^{13}C$ HMBC experiments allowed unequivocal establishment of the structure of **1** as shown. In addition, the presence of a series of minor signals in the ^1H and ^{13}C NMR spectra, both for the isoprenyl and the diketone part of the molecule, demonstrated that about 20% of the compound was present as the β-diketone **1b** (two carbonyls at δ 207.74 and 202.10, with an isolated CH_2 in between at δ 58.38 in the ^{13}C NMR spectrum; the latter corresponded to a two-proton singlet at δ 3.53 in the ^1H NMR spectrum). ^1H and ^{13}C NMR assignments as well as long-range $^1H-^{13}C$ correlations for **1a** and **1b** are listed in Table 1. EIMS of **1** revealed diagnostic fragment peaks at *m*/*z* 167 ($C_{10}H_{15}O_2$), 151 ($C_{11}H_{19}$) (loss of 85 amu), 85 ($C_4H_5O_2$), and 69 (C_5H_9), which are rationalized in Figure 1. Compound **1**, or 8-methyl-5-(3-methyl-2-butenyl)-7-nonene-2,4-dione, for which the trivial name oumarone was adopted, has not been reported before.

The EIMS of compound **2** showed a molecular ion at *m*/*z* 304, while HREIMS indicated a molecular formula of $C_{20}H_{32}O_2$ (*m*/*z* 304.239). From the ^1H and ^{13}C NMR data it was evident that the same β-diketone moiety as in **1** was present, also predominantly in its enolic form (**2a**). The remaining C_{15} part could be assigned to three isoprenyl groups; however, no symmetry (as in **1**) was observed. Whereas for three isoprenyl groups six methyls were expected (apart from C-1), only five appeared to be present. In contrast, an additional CH_2 group occurred at δ 39.79 in the ^{13}C NMR spectrum. This indicated a head-to-tail condensation of two isoprenyl units. Indeed, the ^{13}C NMR assignments for C-6 to C-15 were in agreement with those reported for, for example, the sesquiterpene nerolidol.[3] The chemical shift of the C-14 methyl group was indicative of a trans (or *E*) stereochemistry of the 7,8 double bond. $^1H-^1H$ COSY and $^1H-^{13}C$ HMBC experiments allowed the unequivocal establishment of the structure of **2** as shown. As for **1**, the presence of a series of minor signals in ^1H and ^{13}C NMR spectra demonstrated that about 15% of the compound was present as the β-diketone **2b**. ^1H and ^{13}C NMR assignments as well as long-range $^1H-^{13}C$ correlations for **2a** and **2b** are listed in Table 2. The EIMS of **2** showed characteristic fragment peaks at *m*/*z* 235 ($C_{15}H_{23}O_2$), 167 ($C_{10}H_{15}O_2$), 85 ($C_4H_5O_2$), and 69 (C_5H_9), which are rationalized in Figure 1. Compound **2**, or 8,12-dimethyl-5-(3-methyl-2-butenyl)-7,11-tridecadiene-2,4-dione, for which the trivial name bissaone was adopted, has not been reported before.

The EIMS of compound **3** showed a molecular ion at *m*/*z* 330, while HREIMS indicated a molecular formula of $C_{20}H_{26}O_4$ (*m*/*z* 330.182). In the ^{13}C NMR spectrum four carbonyl signals were present. The $^1H-^1H$ COSY spectrum revealed four spin systems: an isoprenyl group, a $(CH_3)_2$-$CH-CH_2-CH_2$ moiety (saturated isoprenyl), an isolated methyl group, and a CH_2-CH moiety. The presence of only one quaternary unsaturated carbon signal (at δ 113.43), apart from the double bond in the isoprenyl group and the carbonyl functionalities, was in agreement with the presence of a β-diketone in its enolic form, substituted between the two carbonyls. The corresponding CH group in **1** and **2** occurred around δ 100. Detailed analysis of the long-range $^1H-^{13}C$ correlations allowed construction of a partial structure linking together the four fragments mentioned above. Analysis of the long-range correlations observed for the four carbonyl groups, shown in Figure 2, led unequivocally to structure **3**. ^1H and ^{13}C NMR assignments, as well as all HMBC correlations observed, are listed in Table 3. The carbonyl group at C-6 showed a typical chemical shift for an isolated ketone, whereas the three other carbonyls were shifted upfield due to enolization.

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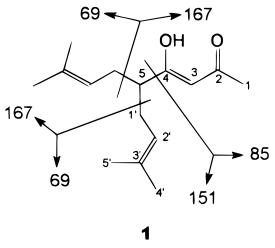
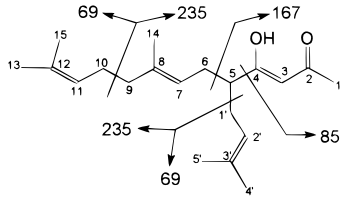
Table 1. NMR Spectral Data for **1**[a]

	Tautomer **1a**			Tautomer **1b**		
position	^{1}H δ, mult.	^{13}C δ	HMBC (C → H)	^{1}H δ, mult.	^{13}C δ	HMBC (C → H)
1	2.05, s	25.15	3	2.05, s	25.15	
2		191.75	1, 3		207.74	1, 3
3	5.45, s	100.00	1	3.53, s	58.38	
4		196.38	3, 5, 1′		202.10	3, 1′
5	2.17, m	49.04	2′, 4′, 5′	2.62, m	53.24	
1′	a: 2.19, m	30.61	2′	2.15 -	29.68	5
	b: 2.26, m			2.30, m		
2′	5.05, m	121.51	1′, 4′, 5′	5.06, m	120.99	5, 4′, 5′
3′		133.45	1′, 4′, 5′		134.15	1′, 4′, 5′
4′	1.67, s	25.76	2′, 5′	1.67, s	25.76	2′, 5′
5′	1.59, s	17.79	2′, 4′	1.59, s	17.79	2′, 4′

[a] Recorded in $CDCl_3$ at 400 MHz.



1



2

Figure 1. Structure and MS fragmentation (*m*/*z*) of **1** and **2** (EIMS).

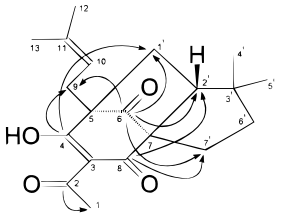
EIMS of **3** showed a rather complex fragmentation pattern. The high mass range showed fragment ions corresponding to the loss of CH_3•, H_2O, CO, and CH_3CO•, at *m*/*z* 315, 312, 302, and 287, respectively. The elimination of H_2O was in agreement with the presence of keto groups, whereas the loss of CH_3CO• supported the presence of an acetyl substituent. This was also indicated by the formation of CH_3CO^+ ions at *m*/*z* 43. In the low mass range, the formation of a $C_5H_9^+$ fragment is energetically favored because the isopentenyl group is attached to a tertiary carbon center and because of resonance stabilization of the $C_5H_9^+$ ions. In contrast to EIMS, negative-ion FABMS showed more specific fragmentation and only revealed two fragment ions at *m*/*z* 219 and 83. A product ion spectrum of $[M - H]^-$ ions, obtained using collision-induced dissociation with argon gas at low kinetic energy (30 eV), showed diagnostic product ions at *m*/*z* 287, 219, and 83, corresponding to the loss of elements of ketene (CH_2═C═O), the combined loss of elements of ketene and C_4H_8, and the formation of $C_4H_3O_2^-$ ions.

The absolute stereochemistry of **3** has not been determined. The C-6 carbonyl bridge between C-5 and C-7 only allows a (5*R*,7*S*)- or a (5*S*,7*R*)-configuration. In the case of a (5*R*,7*S*)-stereochemistry (as shown for **3**), a (2′*S*)-configuration was preferred because of high ring strains observed for the (2′*R*)-isomer when constructing a molecular model. In conclusion, the absolute stereochemistry of **3** is (5*R*,7*S*,2′*S*) or (5*S*,7*R*,2′*R*). The IUPAC name for compound **3**, for which the trivial name aissatone was adopted, with the stereochemistry as shown in Figure 2 but in its tetraketo-form, is (1*S*,5*R*,7*R*)-9-acetyl-4,4-dimethyl-7-(3-methyl-2-butenyl)-tricyclo[5.3.1.0^{1,5}]undecane-8,10,11-trione. This skeleton has not been reported before from nature.

Table 2. NMR Spectral Data for **2**[a]

	Tautomer **2a**[b]		
position	^{1}H δ, mult.	^{13}C δ	HMBC (C→H)
1	2.05, s	25.17	
2		191.78	1, 3, 5
3	5.45, s	100.04	1
4		196.31	3
5	2.18, m	49.04	6, 1′
6	a: 2.18, m	30.54	
	b: 2.25, m		
7	5.05, m	121.50[c]	9, 14
8		137.11	9, 14
9	1.96, m	39.79	10, 14
10	2.04, m	26.72	9
11	5.05, m	121.58[c,d]	9, 13, 15
12		133.41[e]	13, 15
13	1.67, s	25.68[f]	15
14	1.58, s	16.10	
15	1.58, s	17.67[g]	13
1′	a: 2.18, m	30.54	
	b: 2.25, m		
2′	5.05, m	124.24[d]	4′, 5′
3′		131.39[e]	4′, 5′
4′	1.67, s	25.76[f]	5′
5′	1.58, s	17.79[g]	4′

[a] Recorded in $CDCl_3$ at 400 MHz. [b] Tautomer **2b**: ^{1}H NMR δ 3.53 (s, H-3), 2.61 (m, H-5); ^{13}C NMR δ 207.72 (C-2), 58.36 (C-3), 202.06 (C-4), 53.24 (C-5), 120.82, 120.98, 124.11 (C-7, C-11, C-2′), 39.76 (C-9), 26.01 (C-10); HMBC correlations observed between C-2 and H-3, C-4 and H-3, C-4 and H-5. Other signals and HMBC correlations are identical as in **2a**, or not observed because of excessive overlap. [c–g] Assignments bearing the same superscript may be interchanged.



3

Figure 2. Structure and HMBC correlations involving carbonyl groups observed for **3** (C → H).

Biogenetically, the C-1 through C-8 chain in **1**−**3** can be considered as a polyketide consisting of four C_2 units (one acetate and three malonates), substituted at C-5 with two isoprenyl chains, one of which is saturated (C-1′ through C-5′). The C-6′−C-7′ moiety is presumed to be an additional C_2 component originating from malonyl-CoA. This biogenetic hypothesis was supported by the presence of **1** and

Table 3. NMR Spectral Data for **3**[a]

position	^{1}H δ, mult., J	^{13}C δ	HMBC (C → H)
1	2.36, s	32.07	
2		201.67	1
3		113.43	
4		197.11	9b, 1′
5		70.18	9, 12, 13, 1′
6		213.85	9b, 1′, 2′, 7′a
7		76.17	2′, 6′, 7′
8		194.47	2′, 7′
9	a: 2.33, m	27.16	1′b
	b: 2.69, dd, 14.8, 9.4		
10	5.25, m	122.34	9, 12, 13
11		134.32	9, 12, 13
12	1.70, s	26.05	10, 13
13	1.72, s	17.96	10, 12
1′	a: 1.85, m	29.55	9, 2′
	b: 1.90, m		
2′	2.18, dd, 10.1, 5.4	57.32	1′, 4′, 5′, 6′, 7′b
3′		43.11	1′, 2′, 4′, 5′, 6′, 7′b
4′	0.98, s	28.41	2′, 5′, 6′
5′	0.73, s	22.29	2′, 4′, 6′
6′	a: 1.59, m	44.62	4′, 5′, 7′
	b: 1.64, m		
7′	a: 1.88, m	21.69	6′
	b: 2.44, ddd, 13.2, 8.3, 2.5		

[a] Recorded in CD_3OD at 600 MHz.

2, containing three condensed C_2 units (one carbon being removed by decarboxylation), substituted at C-5 with two isoprenyl units or one isoprenyl and one geranyl unit. Alternatively, in **3** the C-7′−C-6′−C-3′−C-4′−C-5′ moiety can be considered as an isoprenyl group as well, with C-1′−C-2′ being the additional C_2 unit. Biogenetically, **3** shows some similarity with humulone and related hop and beer bitter acids, which are prenylated acylphloroglucinols. Phloroglucinol (or 3,5-dihydroxyphenol) consists of three condensed C_2 units.[4] The basic acylphloroglucinol structure is still present in **3**, but, because of the quaternization of C-5 and C-7′, the aromaticity is lost.

Experimental Section

General Experimental Procedures. EIMS, including high-resolution measurements (HREIMS) and FABMS, was performed on a VG70SEQ instrument (Micromass, Manchester, UK), using glycerol as the liquid matrix. 1D ^{1}H and ^{13}C NMR spectra (including APT and DEPT), and 2D ^{1}H−^{1}H COSY, ^{1}H−^{13}C HMQC or HSQC (one-bond correlations), and ^{1}H−^{13}C HMBC (long-range correlations) spectra were recorded on Bruker DRX-400 (400 MHz) and Bruker AM-600 (600 MHz) instruments. UV spectra were recorded on a Uvikon 931 instrument (Kontron). Optical rotations were measured on a Perkin−Elmer model 241 polarimeter.

Plant Material. The roots and stem bark of *Harrisonia abyssinica* Oliv. (Simaroubaceae) were collected around Sérédou (Guinea) in July 1987. The plant was identified at the Department of Botany of the Research Center of Medicinal Plants, Sérédou (Guinea), where a voucher specimen is kept.

Extraction and Isolation. All compounds were obtained from the *n*-hexane extract of powdered stem bark (1 kg) of *H. abyssinica*. After evaporation to dryness under reduced pressure, the organic extract was subjected repeatedly to column chromatography on Si gel eluted with *n*-hexane and a *n*-hexane−$CHCl_3$ gradient. The most lipophilic fraction was purified by a combination of column chromatography on Si gel using the same solvent system and preparative TLC on Si gel, using the upper layer of cyclohexane−MeCN−EtOAc−HCO_2H (80:80:30:1) as the mobile phase. In this way, three lipophilic subfractions (I−III) were obtained, subfraction I being the most lipophilic. Further purification by repetitive preparative TLC on silanized Si gel plates using MeOH−H_2O (1:1 and 8:2) as the mobile phase yielded compounds **1** (24 mg) and **2** (49 mg) from subfraction II and compound **3** (12 mg) from subfraction III. Structures were elucidated by MS and NMR spectroscopy.

Oumarone [8-methyl-5-(3-methyl-2-butenyl)-7-nonene-2,4-dione] (1): obtained as a yellowish oil; UV ($CHCl_3$) λ_{max} 278 nm; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m/z* 236 $[M]^+$ (13), 167 (100), 151 (38), 85 (98), 69 (84); HREIMS *m/z* 236.177 (calcd for $C_{15}H_{24}O_2$, 236.178).

Bissaone [8,12-dimethyl-5-(3-methyl-2-butenyl)-7,11-tridecadiene-2,4-dione] (2): obtained as a yellowish oil; $[\alpha]^{35}_D$ −2.5° (*c* 0.20, $CHCl_3$); UV ($CHCl_3$) λ_{max} 278 nm; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m/z* 304 $[M]^+$ (8), 235 (22), 167 (50), 85 (87), 69 (100); HREIMS *m/z* 304.239 (calcd for $C_{20}H_{32}O_2$, 304.238).

Aissatone [(1*S*,5*R*,7*R*)-9-acetyl-4,4-dimethyl-7-(3-methyl-2-butenyl)-tricyclo [5.3.1.0^{1,5}]undecane-8,10,11-trione] (3): obtained as an amorphous powder; $[\alpha]^{35}_D$ −7.3° (*c* 0.24, MeOH); UV (MeOH) λ_{max} 267 nm; ^{1}H NMR (CD_3OD, 600 MHz), see Table 3; ^{13}C NMR (CD_3OD, 150 MHz), see Table 3; EIMS *m/z* 330 $[M]^+$ (100), 315 (52), 312 (23), 302 (26), 287 (31); FABMS (neg. ion mode) *m/z* 329 $[M - H]^-$, 219, 83; HREIMS *m/z* 330.182 (calcd for $C_{20}H_{26}O_4$, 330.182).

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