Pergamon

PII: S0031-9422(97)00412-3

Phytochemistry, Vol. 47, No. 2, pp. 221–226, 1998

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

SESQUITERPENES FROM SOUTHERN *MAGNOLIA VIRGINIANA*

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(*Received* 3 *March* 1997)

Key Word Index—*Magnolia virginiana*; Magnoliaceae; sesquiterpene lactones; costunolide; parthenolide; sesquiterpene lactol; costunolact-12β-ol; acetal dimer of costunolact-12β-ol.

Abstract—A chemical study of the leaves of the southern evergreen *Magnolia virginiana* provided, besides the known sesquiterpene lactones costunolide, parthenolide and trifloculoside, two new sesquiterpenes, costunolact-12β-ol and its acetal dimer. The molecular structure of the acetal dimer of costunolact-12β-ol was determined by single crystal X-ray diffraction. An annual study of the relative ratios of the major sesquiterpenes in leaves of *M. virginiana* showed $< 10\%$ of costunolide over the 12 month period. In contrast, parthenolide and costunolact-12β-ol varied markedly over the same time period with a maximum of $> 90\%$ of parthenolide in July/August and a minimum of $< 20\%$ in January. However, no lignans typical of the northern variety of *M. virginiana* could be detected in the annual study of the southern variety of this taxon. © 1997 Elsevier Science Ltd

INTRODUCTION

The North American *Magnolia virginiana* L., the type species of the genus *Magnolia*, with the popular names Sweet Bay, Swamp Bay, Swamp Laurel and Swamp Magnolia occurs along the east coast of the United States from Florida and Texas northward to Pennsylvania, New Jersey and locally in eastern Massachusetts [1]. Although a native of the eastern and southeastern United States, *M. virginiana* was introduced to other parts of the world as an ornamental. A southern variety of *M. virginiana* was proposed in 1919 by Sargent as *M. virginiana* var. *australis* [2] with its distribution range from North Carolina to Florida and Texas [2]. It was later recognized by Ashe (1931) as a separate species, *M. australis* [3]. Although, the then leading world authority on the genus *Magnolia* J. E. Dandy, did not concur with Ashe's specific recognition of *M. virginiana* var. *australis* [4], McDaniel re-emphasized in 1966, that *M. virginiana* should be separated into three varieties or possibly into two species [5].

In continuation of our search for biologically active compounds from the genus *Magnolia* [6–8], the chemistry of *M. virginiana* collected in a native forest in Mississippi was investigated. Previously, a chemical study of this species was performed with foliage from potted trees and had resulted in the isolation of several bioactive neolignans: 4,4′-dially-2,3′-dihydroxybiphenyl ether, 3,5′-dially-2′-hydroxy-4-methoxybiphenyl and 5,5′-dially-2,2′-dihydroxybiphenyl [9]. However, our investigation of *M. virginiana* from a native forest in the Ragland Hills, Mississippi provided sesquiterpenes but no neolignans. Therefore, an extensive chemical investigation of this southern variety of *M. virginiana* was performed. Besides the common known germacrolides costunolide (**1**) [6] and parthenolide (**2**) [7, 8, 10], the new costunolact-12β-ol (**3**), and its acetal dimer (**4**) were isolated. In the Mississippi collection of *M. virginiana* the known guaianolide trifloculoside [11, 12] was found as a very minor constituent. The structures of the known and new sesquiterpenes were established by spectroscopic methods, mainly by mass spectral analysis as well as 1D and 2D NMR experiments. The molecular structure of the acetal dimer (**4**) was established by single crystal X-ray diffraction.

Parthenolide (**2**) exhibits a wide spectrum of biological activities including anti-inflammatory [13] and antirheumatoid arthritis activity [14]. It is also the active principle in European feverfew (*Tanacetum parthenium*), which is in popular use for the prophylactic treatment of migraine headaches [15, 16]. Since parthenolide is the major sesquiterpene lactone in European feverfew and *M. grandiflora* [7, 10] and a potent inhibitor of human platelet aggregation and degranulation [17], it is considered to be its major antimigraine

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principle [18]. We showed most recently that parthenolide also inhibits the expression of mitogen-inducible cyclooxygenase (COX-2) and pro-inflammatory cytokines (TNE_{α} and IL-1) in lipopolysaccharide (LPS)-stimulated macrophages [19]. It also suppresses LPS-stimulated protein tyrosine phosphorylation in the murine macrophage cell line (RAW 264.7), which was correlated with its inhibitory effect on the expression of COX-2 and the cytokines, with the mitogen-activated protein kinases (MAPKs) being most strongly inhibited [19]. In this context it is of interest to point out that the Houma Indians, inhabiting the southern marsh lands of the Mississippi delta in which *M. virginiana* occurs, prepared a tincture of this plant for use in chronic rheumatism and as a medicine for treating coughs, colds and fever [1, 20].

Besides the structural arguments, the seasonal variation of the constituents **1–3** within southern *M. virginiana* will be presented in this paper.

RESULTS AND DISCUSSION

Ground leaves of *M. virginiana* were extracted with dichloromethane and chromatography of the crude extract on silica gel resulted in the isolation of costunolide (**1**) and parthenolide (**2**), which were identified by NMR comparison with known standards [6–8, 10], which we had previously isolated from *M. grandiflora* [7]. In addition, the known guaianolide trifloculoside [11, 12], the new costunolact-12β-ol (**3**) and its acetal dimer (**4**) were obtained.

Costunolact-12β-ol (**3**) showed IR absorptions typical of hydroxyl (3391 cm^{-1}) and double bonds (1690 cm^{-1}), respectively. Mass spectral data gave a $[M]^+$ at *m/z* 234 consistent with the formula $C_{15}H_{22}O_2$. The ^1H NMR spectrum of **3** was similar to the spectrum of costunolide (**1**), except for the presence of a broad singlet at δ 5.79 and the upfield shift and smaller coupling constants of the signals for the corresponding olefinic exocyclic methylene protons (H-13a and H-13). This finding together with the absence of a γ-lactone absorption in the IR spectrum and the lack of a carbonyl signal in the ^{13}C NMR spectrum plus the appearance of a methine signal at δ 100.2 by DEPT experiments suggested the presence of a lactol group at C-12 instead of the carbonyl function in costunolide (**1**). Unambiguous evidence was provided by oxidation of the lactol group by air, which provided costunolide (**1**) as shown by ^1H NMR spectral comparison with a standard, thus confirming structure, stereochemistry and conformation of **3**, except at C-12. The stereochemistry of the chiral centre C-12 was tentatively assigned on the basis of differences in chemical shifts of H-6 and H-7 in **1** and **3**, suggesting an β-orientation of the C-12 hydroxyl group. This was supported by irradiation of H-6 in NOE difference experiments which showed no effect on H-12. Complete assignments of the ^1H and ^{13}C NMR spectra of costunolact-12β-ol (**3**) were achieved by 2D homo- and hetero-nuclear experiments, in particular, DEPT, ^1H–^{13}C correlations and COLOC experiments (Table 1). The unambiguous assignments of the quaternary carbons in **3** required the application of COLOC experiments. The carbon signal at δ 138.0 exhibited a

Table 1. ^1H NMR and ^{13}C NMR spectral data of costunolact-12β-ol (**3**) and its acetal dimer **4** ($CDCl_3$ as int. standard)*

Atom	3		4	
	^1H	^{13}C	^1H	^{13}C
1	4.81 *dd* (11, 4.0)	126.5	4.81 *dd* (11, 4.0)	126.5
2α	2.24 *m*†	—	2.24 *m*†	—
2β	2.16 *m*	26.4	2.16 *m*	26.4
3α	1.98 *m*‡	39.4	1.98 *m*‡	39.4
3β	2.24 *m*†	—	2.24 *m*†	—
4	—	138.0	—	138.0
5	4.71 *br d* (9.5)	129.1	4.71 *br d* (9.5)	129.1
6	4.36 *dd* (9.5, 9.5)	79.5	4.36 *dd* (9.5, 9.5)	79.5
7	2.24 *m*†	52.8	2.24 *m*†	52.8
8α	1.61 *m*	—	1.61 *m*	—
8β	1.98 *m*‡	27.1	1.98 *m*‡	27.1
9α	2.43 *m*	—	2.43 *m*	—
9β	2.09 *m*	41.2	2.09 *m*	41.2
10	—	137.7	—	137.7
11	—	152.6	—	152.6
12	5.79 *br s*	100.3	5.79 *br s*	100.3
13	5.12 *d* (2.9)	109.2	5.12 *d* (2.9)	109.2
13′	5.28 *d* (2.6)	—	5.28 *d* (2.6)	—
14	1.37 *s*	16.1	1.37 *s*	16.1
15	1.67 *s*	17.2	1.67 *s*	17.2

* ^1H, (400 MHz) and ^{13}C NMR (100 MHz) spectra were assigned on the basis of 2D COSY, ^1H–^{13}C HETCOR and COLOC experiments.

†,‡ signals are overlapped.

^2J coupling with Me-15, as did the signal at δ 137.7 with Me-14, allowing the former carbon signal to be assigned to C-4 and the latter to C-10. Similarly, the carbon absorption at δ 152.6 could be assigned to C-11 by its correlation to H-7.

Chromatography by prep. TLC on silica gel provided both the C-12 β-epimer **3** and α-epimer **3a** as minor and major components, respectively. It was also observed by ^{1}H and ^{13}C NMR that the natural C-12 β-epimer (**3**) converted slowly into the more stable α-epimer of costunolact-12β-ol (**3**).

Upon storage of the crude dichloromethane extract of *M. virginiana* under refrigeration and subsequent chromatography on silica gel provided from nonpolar VLC fractions the dimer of costunolact-12β-ol (**4**), which formed colourless crystals when recrystallized from hexane. Its structure was established by ^{1}H and ^{13}C NMR spectral analysis as well as single crystal X-ray diffraction. The ^{13}C NMR and DEPT experiments of **4** exhibited 15 carbon signals with five methine absorptions including two olefinic and one oxygenated carbon, five methylene signals including one olefinic carbon, two methyl groups and three olefinic quaternary carbons. However, the molecular ion in the FABMS at m/z 451 $[M+1]^+$ revealed that compound **4** was consistent with the empirical formula $C_{30}H_{42}O_3$. Inspection of the ^{1}H NMR spectrum of **4** showed that it was essentially identical with that of costunolide (**1**), except for the presence of the broad singlet at δ 5.79 and minor changes in chemical shifts. In addition, the absence of a hydroxyl absorption in the FT-IR spectrum and the lack of carbonyl signal in the ^{13}C NMR spectrum, as well as the appearance of a methine signal at δ 100.3 typical for a ketal carbon suggested the possibility of a lactol dimer being connected at C-12 of the two lactol moieties. Single crystal X-ray diffraction data of **4** supported this structure, indicating a β-oriented oxygen connecting the two lactol ring systems, as shown in Fig. 1.

The acetal dimer of costunolact-12β-ol (**4**) must be derived from costunolact-12β-ol (**3**) by acid-mediated acetalization, possibly during silica gel chromatography. Reverse-phase HPLC analysis of freshly obtained crude extracts of *M. virginiana* showed that

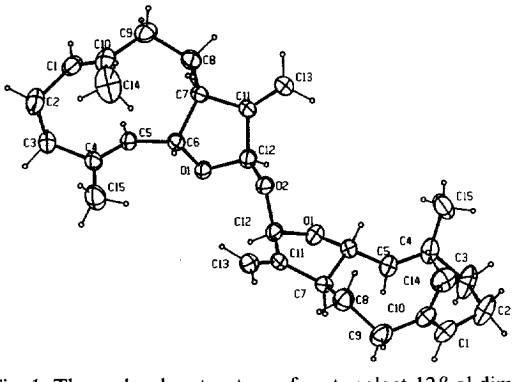

Fig. 1. The molecular structure of costunolact-12β-ol dimer (**4**).

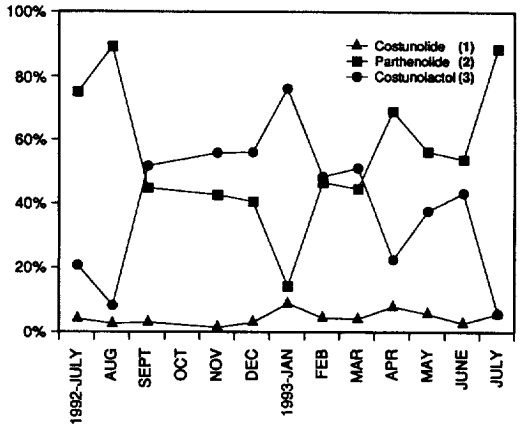

Fig. 2. The annual variation of sesquiterpenes **1**–**3** in leaves of southern *M. virginiana*.

4 was present only as a very minor component, suggesting that **4** represents an artefact, most likely formed from **3** during the chromatography procedure and/or during storage of the crude extracts.

The previous isolation of neolignans from *M. virginiana* [9] and our findings that the southern variety of this taxon gave sesquiterpenes lactones **1** and **2** and the lactol **3** could be due to seasonal variations in the secondary metabolites of this species. Therefore, a chemical analysis of crude extracts of monthly collections obtained from leaves of a single plant of southern *M. virginiana* was carried out during the period between July 1992 and July 1993.

Quantitative analyses of constituents **1**–**3** in freshly obtained crude extracts of monthly collections of *M. virginiana* were performed by RP-HPLC. The elution order of the sesquiterpenes was established by the comparison with standards of costunolide (**1**), parthenolide (**2**) and costunolact-12β-ol (**3**). The variation of these three sesquiterpenes in leaves of *M. virginiana* over a one-year period is shown in Fig. 2. The results clearly demonstrated that the southern variety of *M. virginiana* produces sesquiterpenes **1**–**3**, but no lignans could be detected. In contrast, chromatograms of foliage extracts from *M. virginiana* ornamentals, planted on the Louisiana State University Campus and the LSU Hilltop Arboretum in Baton Rouge, Louisiana, which were most likely obtained from commercial nursery supplies, indicated the presence of lignans, but no sesquiterpenes, which is in agreement with results reported by Nitao *et al.* [9].

In conclusion, our studies clearly illustrate that native southern *M. virginiana* only produces sesquiterpenes, with major variations of the ratios of **2** and **3** during the growing cycle of the plant. The above findings are also of considerable importance, in particular in ecological studies of taxa of *M. virginiana* from different regions and their interaction with other organisms [21]. Based on its secondary metabolites, the southern variety of *M. virginiana* seems to be closely aligned with *M. grandiflora*, which typically contains, besides other sesquiterpene lactones, costu-

Table 2. Fractional atomic coordinates and equivalent isotropic thermal parameteres (Å²)*

	x	y	z	B_{eq}
O1	0.5800(2)	0.2909(2)	0.54601(5)	5.26(4)
O1′	0.4173(2)	0.2502(2)	0.66667(6)	5.98(4)
O2	0.5384(2)	0.3878(2)	0.61637(6)	5.50(4)
C1	0.8341(4)	0.5163(3)	0.4109(1)	7.15(8)
C1′	0.1388(5)	0.3207(3)	0.8178(1)	8.06(9)
C2	0.7025(5)	0.5203(4)	0.3784(1)	8.7(1)
C2′	0.2353(5)	0.2464(4)	0.8504(1)	10.4(1)
C3′	0.3337(6)	0.1534(3)	0.8227(1)	10.6(1)
C3	0.5900(4)	0.4182(3)	0.3905(1)	6.88(8)
C4	0.5467(3)	0.4247(3)	0.44032(9)	5.84(7)
C4′	0.4128(4)	0.2226(3)	0.78478(9)	7.19(8)
C5′	0.3458(4)	0.2313(3)	0.74479(9)	6.04(7)
C5	0.6330(3)	0.3678(2)	0.47037(8)	4.85(6)
C6′	0.3751(3)	0.3198(3)	0.70715(8)	5.14(6)
C6	0.6458(3)	0.3928(2)	0.52000(9)	4.65(6)
C7′	0.2384(3)	0.3955(3)	0.69077(9)	5.42(7)
C7	0.8094(3)	0.3972(3)	0.53817(9)	4.95(6)
C8′	0.1792(4)	0.5060(3)	0.7188(1)	7.44(9)
C8	0.9009(4)	0.5173(3)	0.5318(1)	6.47(8)
C9′	0.0816(4)	0.4795(3)	0.7608(1)	8.15(9)
C9	0.9666(4)	0.5454(3)	0.4842(1)	7.38(9)
C10′	0.1634(4)	0.4329(3)	0.8017(1)	6.68(8)
C10	0.8547(5)	0.5843(3)	0.4487(1)	7.29(9)
C11′	0.2913(3)	0.4314(3)	0.64370(9)	5.40(6)
C11	0.7843(3)	0.3619(3)	0.58704(9)	5.24(6)
C12′	0.3999(4)	0.3316(3)	0.62911(9)	5.65(7)
C12	0.6317(3)	0.3035(2)	0.59084(9)	5.30(6)
C13′	0.2583(4)	0.5291(4)	0.6188(1)	7.77(9)
C13	0.8721(4)	0.3757(4)	0.6224(1)	7.77(9)
C14	0.7592(7)	0.6966(3)	0.4610(2)	12.0(2)
C14′	0.2799(5)	0.5225(3)	0.8201(1)	8.9(1)
C15	0.4185(4)	0.5100(4)	0.4519(1)	9.9(1)
C15′	0.5513(5)	0.2954(4)	0.7972(1)	9.7(1)

* Hydrogen atoms were refined isotropically, and B_{iso} values are given.
$B_{eq} = (8\,\Pi^2/3)\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* a_i \cdot a_j$.

nolide (**1**) and parthenolide (**2**) as common constituents [7, 10]. Finally, these chemical results again invite the question on the taxonomic status of the various taxa within *M. virginiana*.

X-Ray data of the dimer of costunolact-12β-ol (**4**)

The crystal structure of **4** is illustrated in Fig. 1, and its coordinates are tabulated in Table 2. A search of the Cambridge Crystallographic Database [22] yielded no other structure determinations of sesquiterpene lactol dimers. The conformation of costunolactol dimer (**4**) has approximate symmetry C_2, with endocyclic torsion angles of the two halves differing by an average of 4.9°. The largest differences are 11.5° for C7-C11-C12-O1 and 10.2° for C4-C5-C6-C7. The O2-C12-C11-C13 torsion angle differs by 15.1° between the two halves. The conformation of the ten-membered rings are typical of other costunolide derivatives, agreeing well with those found in costunolide [23], its $AgNO_3$ complex [24], herbolide A [25], 8α-isobutyroxycostunolide [26], and a costunolide diepoxide [27].

EXPERIMENTAL

General. NMR: 400 (^{1}H) and 200 (^{13}C) Hz; IR: film on KBr plates; UV and CD: hexane as solvent; analytical HPLC: 5 μm Spherisorb S_5C_8 (Phase Sep) and Spherisorb ODS-2 (Alltech) reverse-phase column (250 × 4.6 mm); vacuum liquid chromatographic (VLC) [28]: silica gel (MN Kieselgel G); TLC: precoated MN Sil-G 25 UV254 plates (thickness 0.25 mm and 1.0 mm).

Plant material. The leaves of *M. virginiana* L. were collected in the Ragland Hills, Forest County, Mississippi, U.S.A. on 30 April 1991. A voucher specimen is deposited at the Louisiana State University Herbarium (N. H. Fischer and H. D. Fischer no. 414). The location for the collection of leaves for the annual study of a single native southern *M. virginiana* plant from July, 1992 to July, 1993 was replanted on the

property of 1025 Highland Park Drive, Baton Rouge, East Baton Rouge Parish, Louisiana. The voucher specimen (N. H. Fischer and H. D. Fischer, no. 467) is deposited at the Louisiana State University Herbarium.

Extraction and isolation. The air-dried leaves (940 g) were ground and extracted with CH_2Cl_2 at room temp. for 24 hr to give 17 g of crude extract. Part of the extract (11.6 g) was chromatographed by VLC on silica-gel using *n*-hexane–EtOAc mixt. of increasing polarity, 65 frs of 25 ml each being collected. The combined frs 20–21 were recrystallized from hexane to afford 42 mg of colourless crystal of dimer **4**. Frs 22–25, after rechromatography by prep. TLC with CH_2Cl_2–hexane (1:1), gave additional **4**. Frs 27–30 provided costunolide (**1**) and frs 35–37, after prep. TLC with CH_2Cl_2–hexane (4:1), afforded 105 mg of lactol **3**, which was also present in subsequent frs. Further purifications were carried out by prep. HPLC using H_2O–CH_3CN (3:1). Frs 38–44 gave parthenolide (**2**). A more polar fr. using hexane–EtOAc (2:1) gave after reverse-phase prep. HPLC with MeCN–H_2O (1:1), 1 mg of **5**.

Costunolact-12β-ol (**3**). $C_{15}H_{22}O_2$, colourless oil. IR ν_{max}^{KBr} cm^{-1}: 3393 (OH), 1665 (C=C); UV λ_{max}^{MeOH} nm (log ε): 217 (sh); EIMS (70 eV) *m/z* (rel. int.): 234 $[M]^+$, 219 $[M-Me]^+$, 201, 165, 145, 135, 121, 105, 81, 41; CD (hexane; *c* 3.33×10^{-4} M): $[\theta]_{192}$ −14458, $[\theta]_{219}$ +26012; ^1H and ^{13}C NMR: Table 1.

Costunolact-12β-ol dimer (**4**). $C_{30}H_{42}O_3$, colourless crystals; decomposed upon heating. IR ν_{max}^{KBr} cm^{-1}: 1690 (C=C); UV λ_{max}^{MeOH} nm (log ε): 216 (sh); FABMS (ionization gun is Cs, acceleration voltage is 6 kV; matrix is NBA) *m/z* (rel. int.): 451 $[M+1]^+$ (5.5), 423 $[M\text{-}CO+1]^+$ (2.0), 369, 303, 285, 267, 233, 217, 199; CD (hexane; *c* 1.66×10^{-4} M): $[\theta]_{193}$ −4040, $[\theta]_{220}$ +34179; ^1H and ^{13}C NMR: Table 1.

Annual study of terpenes **1–3** *by HPLC analysis.* HPLC analyses were performed on a Hewlett-Packard 1090 liquid chromatograph equipped with a diode array detector. Detection channels were set at 230, 254 and 264 nm with a bandwidth of 8 nm. Chromatograms were recorded and analysed on a Hewlett-Packard HPLC Chemstation (Series 300 computer). The column was a 5 μm Spherisorb S_5C_8 (Phase Sep) and Spherisorb ODS-2 (Alltech) reverse-phase column (250 × 4.6 mm) coupled to a Hewlett-Packard 1090 HPLC system with diode array detection. Analyses were performed at room temp. with a flow rate of 1.0 ml min^{-1} and MeOH–H_2O for isocratic sepns. The samples for the HPLC analysis were obtained after fresh leaves were extracted with CH_2Cl_2 followed by evapn of the solvent. The residue was dissolved in MeOH and filtered through a cartridge of reverse-phase silica gel to remove chlorophyll; then a millipore filter, finally diluted to a certain amount of MeOH.

X-ray data of costunolact-12β-ol dimer (**4**). Recrystallization of the dimer of costunolact-12β-ol (**4**) in hexane afforded colourless crystals. A crystal of dimensions 0.48 × 0.37 × 0.30 mm was used for data collection on an Enraf-Nonius CAD4 diffractometer equipped with CuK_α radiation (λ = 1.54184 Å), and a graphite monochromator. The crystal was sealed in a capillary during data collection. Crystal data are: $C_{30}H_{42}O_3$, M_r = 450.7, orthorhombic space group $P2_12_12_1$, a = 8.8884(4), b = 10.6085(7), c = 29.275(3) Å, V = 2760.4(6) Å^3, Z = 4, d_c = 1.084 g cm^{-3}, T = 24°. Intensity data were measured by ω-2θ scans of variable rate. An octant of data was collected within the limits $2 < \theta < 75°$, and second octant within $2 < \theta < 60°$. Data reduction included corrections for background, absorption, decay (6.8% of original intensities) Lorentz, and polarization effects. Absorption effects (μ = 5.0 cm^{-1}) were based on ψ scans, with minimum relative transmission coefficient 91.6%. Of 4831 unique data, 3425 had $I > 3\sigma(I)$ and were used in the refinement.

The structure was solved by direct methods using SHELXS [29] and refined by full-matrix least squares based on F with weights $w = \sigma^{-2}(F_o)$, using the Enraf-Nonius MolEN programmes [30]. Nonhydrogen atoms were refined anisotropically, while H atoms were included in calculated positions and not refined. An extinction coefficient refined to a final value of $2.07(11)\times10^{-6}$. Convergence was achieved with R = 0.050, R_w = 0.055 using 299 variables, with maximum residual electron density 0.22 eÅ^{-3}. Bond distances bond angles and torsion angles, as well as anisotropic displacement parameters are given in supplementary material.

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