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Article

Constituents of *Bupleurum praealtum* and *Bupleurum veronense* with Potential Immunomodulatory Activity

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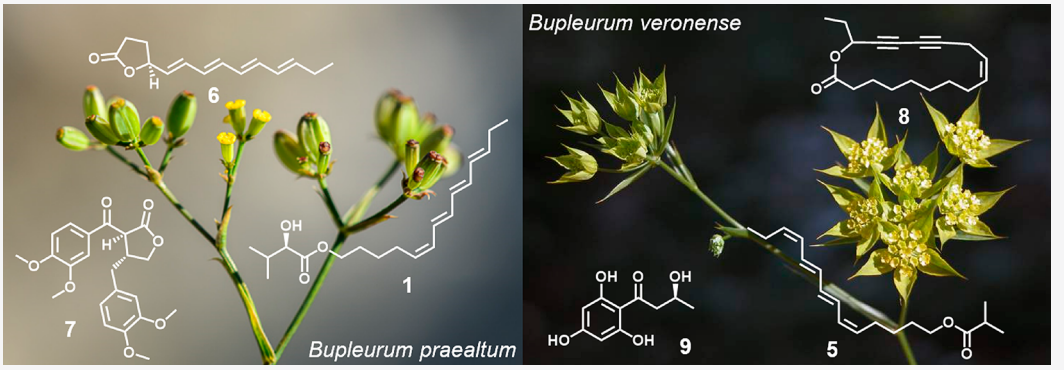

ABSTRACT: In this investigation, chromatographic separations of the diethyl ether extracts of two European annual *Bupleurum* taxa, *B. praealtum* and *B. veronense*, yielded nine new natural products, namely, a series of esters of stereoisomeric tetradeca-5,7,9,11-tetraen-1-ols (**1**−**4** and **8**), a tetra-unsaturated γ-tetradecalactone (**5**), a dibenzylbutyrolactone lignan (7-oxoarcitin, **6**), a falcarinol-related 17-membered macrolide (**7**) possessing a conjugated diyne-system, and an acylphloroglucinol derivative (**9**). All these new compounds were fully characterized by NMR, IR, UV, MS, and optical rotation measurement, including ^{1}H NMR full spin spectral simulation, whereas the absolute configurations of **1**, **5**, and **9** were determined via chemical correlations and NMR analysis of Mosher esters. The in vitro potential immunomodulatory activities of **1**, **4**, **5**, and (+)-arcitin were assessed by determining their effects on the functional properties of isolated rat splenocytes and peritoneal macrophages. The results obtained support the known immunomodulatory ethnomedicinal usage of *Bupleurum* species.

Species of the genus *Bupleurum* L. (Apiaceae) have been utilized by humans for more than two millennia,[1] predominantly as medicinal plants, with the best-documented species being *B. chinense* DC., which is native to East Asia. Due to the incorporation of its dried roots in several major multicomponent traditional Chinese medicines, targeting conditions related to the immune system, *B. chinense* has been in the focus of prior phytochemical and biological work, which resulted in the discovery of the potent immunomodulatory proapoptotic saikosaponins.[2] Other members of the genus, which encompasses ca. 190 Northern Hemisphere Old World species, have been investigated sporadically and have yielded new structures displaying an array of biological activities.[1] Among the isolated secondary metabolites, polyketides (polyacetylenes),[3,4] terpenoids (saponins),[2,5] and shikimate metabolites (lignans and coumarins)[1,6] were the most commonly reported. Many of the polyacetylenes found have been related to the toxic polyunsaturated alcohol falcarinol,[3] while dibenzylbutyrolactone derivatives have been the most frequently encountered lignans, accompanied by arylnaphthalenes, aryltetralinelactones, and tetrahydrofurofurans.[1] Some of the isolated constituents have been demonstrated to affect the viability and functional properties of cells of the immune system (macrophages), consistent with the ethnobotanical uses of *Bupleurum* species.[1]

In Europe, 33 annual species of the genus *Bupleurum* belong to two sections: *Bupleurum* and *Aristata*.[7] Fifteen *Bupleurum* taxa can be commonly found in Serbia, typically on mountain slopes at elevations of up to 2000 m above sea level, whereas some other Balkan *Bupleurum* taxa grow spontaneously even on the sea coastline.[8] Two annual taxa from the section *Aristata*, *B. praealtum* L. (subsection *Juncea*)[7] and *B. veronense* Turra (subsection *Aristata*),[7] are good representatives of these two habitats, respectively. While the probable European

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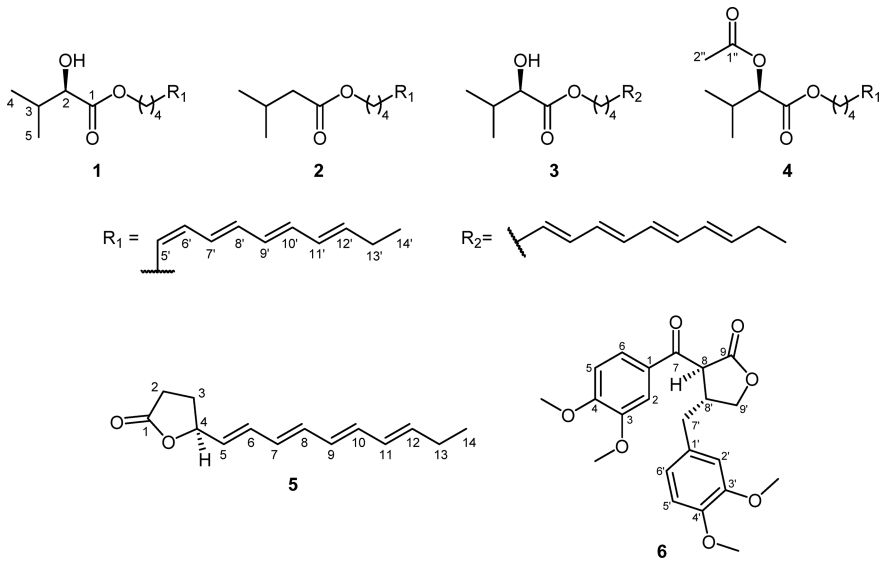

Figure 1. Structures of compounds **1**−**6** isolated from *B. praealtum*.

endemic species, *B. veronense*, has never been previously phytochemically studied, only the essential oil of *B. praealtum* has been analyzed previously.[9] The leaves of *B. veronense* were used against toothache,[10] suggesting a possible anti-inflammatory action.

^{1}H NMR spectra offer a plethora of structural information; however, the reproducibility of the data, permitting dereplication, is more than occasionally hampered by low spectral dispersion and the incidence of higher-order effects.[11,12] ^{1}H NMR iterative full spectral simulation represents a valuable tool for complete spectral interpretation,[13] adequate precision, and unambiguous ^{1}H NMR-driven dereplication.[12,14] In extended unsaturated aliphatic chains, due to local pseudo-symmetry, pairs of protons of isolated and conjugated double bonds are almost isochronous, producing highly complex resonances. Such slight differences in chemical shifts and additional accidental signal isochronicity make dereplication a significant endeavor.[12,15]

This study aimed to investigate, chemically, the constituents of two Balkan species, *B. praealtum* and *B. veronense*. Extracts of these species were subjected to various chromatographic separations, and the structures of the obtained pure constituents were elucidated by spectroscopic means and chemical transformations. Structural elucidation and complete NMR spectroscopic assignments were conducted using iterative ^{1}H NMR full spectral simulations. This resulted in the identification of nine new compounds: four polyunsaturated esters (**1**−**4**), one lactone (**5**), and one lignan (**6**) from *B. praealtum* and falcarinol-related 17-membered macrolide (**7**), one polyunsaturated ester (**8**), and acylphloroglucinol derivative (**9**) from *B. veronense*. Compounds from *B. praealtum*, available in sufficient quantity (**1**, **4**, **5**, and (+)-arcitin), were evaluated for their immunomodulatory activity by assessing their effect on isolated rat splenocytes and peritoneal macrophages. The absolute configurations of three new compounds, **1** and **5** from *B. praealtum* and **9** from *B. veronense*, were determined via the preparation of the corresponding Mosher esters, as well as by comparison of spectroscopic data of the synthesized enantiopure model compounds.

RESULTS AND DISCUSSION

The initial GC−MS analysis of the crude Et_2O extract of the dry umbels of *B. praealtum* revealed the presence of one major constituent (**1**, Figure 1) with a molecular ion at *m*/*z* 306 in its MS. HRMS disclosed the molecular formula of **1** to be $C_{19}H_{30}O_3$, while the IR absorptions at 3100−3500 (max at 3334) and 1727 cm^{-1} suggested the presence of alcohol and ester functions, respectively. Among the 19 resonances observable in the ^{13}C NMR spectrum of **1** (Table 1), an ester carbonyl at 175.0 ppm (C-1) and two oxygenated sp^3-hybridized carbon signals at 65.4 (C-1′) and 74.9 (C-2) ppm

Table 1. ^{13}C NMR Spectroscopic Data (100.6 MHz, $CDCl_3$) of Compounds 1, 3−5, and 8

	1	4	3	8		5
position	δ_C				position	δ_C
1, C	175.0	170.0	175.1	177.3	*a*	*a*
2, CH	74.9	77.0	75.0	34.2	*a*	*a*
2-OH	*a*	*a*	*a*	*a*	*a*	*a*
3, CH	32.2	30.1	32.2	19.1	*a*	*a*
4, CH_3	15.9	17.4	16.0	19.1	*a*	*a*
5, CH_3	18.8	18.9	19.0	*a*	*a*	*a*
1′, CH_2	65.4	65.1	65.5	64.2	1, CH	177.2
2′, CH_2	28.1	28.2	28.1	28.4	2, CH_2	28.8
3′, CH_2	25.9	26.00	25.6	26.1	3, CH_2	29.0
4′, CH_2	27.3	27.5	32.3	27.6	4, CH	80.8
5′, CH	131.1	131.5	133.8	134.8	5, CH	128.9
6′, CH	129.50	129.5	129.59	129.4	6, CH	133.6
7′, CH	127.1	127.3	132.1	128.4	7, CH	135.1
8′, CH	133.3	133.3	131.36[b]	133.3	8, CH	130.04
9′, CH	130.7	130.9	131.43[b]	132.8	9, CH	129.98
10′, CH	133.5	133.5	132.9	128.0	10, CH	135.6
11′, CH	129.53	129.6	129.63	128.2	11, CH	129.4
12′, CH	137.3	137.4	136.9	132.0	12, CH	138.5
13′, CH_2	25.9	26.05	26.0	21.4	13, CH_2	26.1
14′, CH_3	13.5	13.7	13.6	14.4	14, CH_3	13.6
1″, C	*a*	171.0	*a*	*a*		*a*
2″, CH_3	*a*	20.8	*a*	*a*		*a*

[a]No carbon or no position. [b]Assignment can be interchanged.

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Table 2. ^{1}H NMR Spectroscopic Data (400 MHz, $CDCl_3$) of Compounds 1, 3−5, and 8

	1	4	3	8		5
position	δ_H[a], (J[b] in Hz)				position	δ_H[a], (J[b] in Hz)
1	[c]	[c]	[c]	[c]		[c]
2	4.04, dd (6.0, 3.4)	4.82, d (4.7)	4.03, dd (6.0, 3.6)	2.54, t (6.9)		[c]
OH-2	2.81, d (6.0)	[c]	2.81, d (6.0)	[c]		[c]
3	2.07, qqd (7.0, 6.8, 3.4)	2.22 qqd (7.0, 6.8, 4.7)	2.07, qqd (7.0, 6.8, 3.4)	1.16, d (6.9)		[c]
4	0.86, d (7.0)	0.98, d (7.0)	0.86, d (7.0)	1.16, d (6.9)		[c]
5	1.03, d (6.8)	1.00, d (6.8)	1.03, d (6.8)	[c]		[c]
1′a	4.21, dt[d] (−10.8, 6.6)	4.16, ddd (−10.6, 7.0, 6.5)	4.20, dt[d] (−10.7, 6.6)	4.07, t (6.6)		[c]
1′b	4.17, dt[d] (−10.8, 6.6)	4.14, ddd (−10.6, 7.0, 6.5)	4.17, dt[d] (−10.7, 6.6)			[c]
2′a	1.70, dddt[d] (−13.8, 8.0, 7.2, 6.6)	1.68, ddddd (−13.8, 9.0, 7.0, 6.5, 6.0)	1.70, dt[d]t[d] (−13.8, 7.2, 6.6)	1.67, tt (7.6, 6.6)	2a	2.57, ddd (−17.5, 9.5, 9.4)
2′b	1.68, dddt[d] (−13.8, 8.0, 7.2, 6.6)	1.67, ddddd (−13.8, 9.0, 7.0, 6.5, 6.0)	1.68, dt[d]t[d] (−13.8, 7.2, 6.6)		2b	2.54, ddd (−17.5, 9.2, 4.6)
3′a	1.48, ddt[d]d (−13.3, 8.0, 7.6, 7.2)	1.47, ddt[d]d (−13.3, 9.0, 7.6, 6.0)	1.48, dt[d]t[d] (−13.3, 7.6, 7.2)	1.47, quint[d] (7.6)	3a	2.41, ddddd (−13.0, 9.5, 6.8, 4.6, 0.7)
3′b	1.47, ddt[d]d (−13.3, 8.0, 7.6, 7.2)	1.46, ddt[d]d (−13.3, 9.0, 7.6, 6.0)	1.46, dt[d]t[d] (−13.3, 7.6, 7.2)		3b	2.01, dddd (−13.0, 9.4, 9.2, 7.7)
4′a	2.24, ddt[d]d (−17.2, 7.7, 7.6, −1.3)	2.24, ddt[d]d (−17.2, 7.7, 7.6, −1.3)	2.15, dt[d]dd (−17.2, 7.6, 6.9, −1.3)	2.24, td (7.6, −1.4)	4	5.00, ddddd (7.7, 7.0, 6.8, 0.7, −0.7)
4′b	2.23, dt[d]dd (−17.2, 7.6, 7.1, −1.4)	2.23, dt[d]dd (−17.2, 7.6, 7.1, −1.4)	2.13, dt[d]dd (−17.2, 7.6, 6.9, −1.4)			
5′	5.39, dddd (10.3, 7.7, 7.1, 1.1)	5.40, dddd (10.3, 7.7, 7.1, 1.1)	5.65, dt[d]d (14.6, 6.9, −0.7)	5.46, dtd (11.2, 7.6, −1.1)	5	5.65, dddd (15.4, 7.0, 0.7, −0.7)
6′	6.05, dddd (11.3, 10.3, −1.4, −1.3, −0.7)	6.05, ddddd (11.3, 10.3, −1.4, −1.3, −0.7)	6.08, ddt[d]d (14.6. 11.3, −1.3, −0.7)	6.06, ddtd (11.2, 10.9, −1.4, −0.7)	6	6.34, dddtd (15.4, 11.1, −0.9, 0.7, −0.7)
7′	6.43, dddd (14.5, 11.3, 1.1, −0.7)	6.44, dddd (14.5, 11.3, 1.1, −0.7)	6.16, ddd (15.0, 11.3, −0.7)	6.50, dddd (15.4, 10.9, −1.1, −0.7)	7	6.18, ddt[d]t[d] (14.8, 11.1, 0.7, −0.7)
8′	6.224, dddd (14.5, 11.2, −0.8, −0.7)	6.224, dddd (14.5, 11.2, −0.8, −0.7)	6.14, ddd (15.0, 11.0, −0.7)	6.27, ddt[d] (15.4, 10.9, −0.7)	8	6.30, ddddd (14.8, 10.7, −0.9, 0.7, −0.7)
9′	6.19, ddt[d] (15.5, 11.2, −0.7)	6.19, ddt[d] (15.5, 11.2, −0.7)	6.14, ddd (15.0, 11.0, −0.7)	6.26, ddt[d] (15.4, 10.9, −0.7)	9	6.15, ddt[d]d (14.3, 10.7, 0.7, −0.7)
10′	6.216, ddt[d] (15.5, 10.8, −0.8)	6.221, ddt[d] (15.5, 10.8, −0.8)	6.15, ddd (15.0, 11.0, −0.7)	6.47, dddd (15.4, 10.9, −0.8, −0.7)	10	6.26, dddt[d] (14.3, 10.4, 0.7, −0.7)
11′	6.08, ddt[d]d (15.0, 10.0, −1.4, −0.7)	6.09, ddt[d]d (15.0, 10.8, −1.4, −0.7)	6.08, ddtd (14.5, 11,0, −1.4, −0.7)	6.01, ddtd (11.3, 10.9, −1.4, −0.7)	11	6.09, ddt (15.2, 10.4, −1.3)
12′	5.76, dtd (15.0, 6.8, −0.8)	5.77, dtd (15.0, 6.8, −0.8)	5.75, dt (14.5, 7.1)	5.43, dtd (11.3, 7.7, −0.8)	12	5.82, dtdd (15.2, 6.7, 0.7, −0.7)
13′	2.13, qdd (7.5, 6.8, −1.4)	2.13, qdd (7.5, 6.8, −1.4)	2.13, qd (7.5, 7.1, −1.4)	2.22, dqd (7.7, 7.5, −1.4)	13	2.14, qdd (7.4, 6.7, −1.3)
14′	1.02, t (7.5)	1.02, t (7.5)	1.01, t (7.5)	1.01 t (7.5)	14	1.02, t (7.4)
2″	[c]	2.15, s	[c]	[c]		[c]

[a]Chemical shift and coupling constant values were inferred from ^{1}H homoselective decoupling experiments and spin simulation (Figure S1.1.1, Supporting Information). [b]$|J| < 0.7$ values are not listed for the sake of easier comparison. [c]No proton or no position. [d]Pseudo, i.e., a pseudo multiplet. For example, a doublet of doublets but with two similar J values would likely appear as a pseudotriplet.

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further strengthened the existence of the mentioned functional groups. The diastereotopic hydrogens from the C-1′ methylene group appeared at δ_H 4.21 (H-1′a) and 4.17 (H-1′b) in the ^{1}H NMR (Table 2) and correlated through three bonds with C-1 (Figure S1.5, Supporting Information), revealing the presence of a proximal chiral center in **1**, which is in agreement with the observed optical rotation of **1** (+64.3, *c* 1, MeOH). The center was positioned α to the ester group based on the gHMBC interaction of C-1 and a doublet of doublets (J = 6.0, 3.4 Hz) at δ_H 4.05 (H-2) that coupled with a methyne hydrogen (δ_H 2.07) from an isopropyl group and the OH proton (δ_H 2.81, exchanged upon a D_2O shake experiment). The coupling with an exchangeable proton and the gHMBC interaction of OH to C-1 demonstrated the existence of an intramolecular hydrogen bond. Based on this, **1** was concluded to be an ester of 2-hydroxy-3-methylbutanoic acid and a tetra-unsaturated aliphatic primary C_{14}-alcohol. The ^{1}H NMR spectrum (Figure S1.1, Supporting Information) displayed, along with the signals of two diastereotopic methyl groups (δ_H 0.86 and 1.03, Table 2) from the acid part, one additional methyl signal as a nonvirtually coupled triplet at δ_H 1.02 (J = 7.5 Hz) implying that the unsaturated functionalities were near the ω-end of the alcohol and that **1** did not contain any further branching. All four unsaturations were located in four double bonds, apparent from the 8 sp^2-CH ^{13}C NMR/DEPT-90 signals (Table 1), which were mutually conjugated based on the characteristic absorption maxima for conjugated tetraenes noted in the UV spectrum of **1**.[16] Their position in the chain was straightforwardly determined to be at carbons C-5′, C-7′, C-9′, and C-11′ from gHMBC (Figure S1.5, Supporting Information) and gradient ^{1}H−^{1}H COSY spectra; i.e., there is an ethyl group on the ω-end of the alcohol.

The geometries of C-5′−C-6′ and the C-11′−C-12′ double bonds were concluded to be *Z* and *E*, respectively, based on the observable proton−proton ($J_{H\text{-}5'-H\text{-}6'}$ = 10.3 and $J_{H\text{-}11'-H\text{-}12'}$ = 15.0 Hz) and proton−carbon coupling constant values (Figure S1.6, Supporting Information). The ^{1}H-coupled ^{13}C NMR and ^{1}H selective homodecoupling experiments, followed by manual ^{1}H full spin spectral simulation[17] (Figure S1.1.1, Supporting Information), enabled the determination of the geometry (*E*,*E*) of the two inner double bonds and the full assignment of its NMR signals (Tables 1 and 2). A comparison with the NMR data on the closely related methyl parinarate isomers[16] (Tables S1 and S2, Supporting Information) further corroborated the (5*Z*,7*E*,11*E*,13*E*)-configuration of **1**. It should be stressed that the full spectral, structural, and stereochemical assignments could not be achieved solely by a conventional (including 2D NMR) elucidation approach but was possible though ^{1}H full spectral simulation that is essential for the dereplication of polyunsaturated aliphatic chains.[12] The NMR parameters obtained during the simulations, in this case and all others where this approach was applied, are given, to the relevant number of decimal points (i.e., precision; Tables S1−S5, Supporting Information).[12]

The absolute configuration of the chiral center at C-2 in **1** was determined via a comparison of the ^{1}H NMR data of a prepared Mosher ester of (*R*)-MTPA and **1** with the corresponding data for the diastereomerically pure (*R*)-MTPA-esters of two synthesized model compounds, methyl 2-hydroxy-3-methylbutanoates, (*R*)-**1a** and (*S*)-**1a**. The differentiation of the diastereomeric esters was most notable in the case of the chemical shifts of the geminal methyl groups C-4 and C-5, and *ortho*-hydrogens on the benzene ring, as well as the methoxy group (Figure S1.7, Supporting Information). An excellent correspondence of the data for (*R*)-MTPA-**1** and (*R*)-MTPA-(*R*)-**1a** (Table S3 and Figure S1.7, Supporting Information), and a considerable difference from the signals of (*R*)-MTPA-(*S*)-**1a** (Table S3 and Figure S1.7, Supporting Information), enabled compound **1** to be assigned as (5*Z*,7*E*,9*E*,11*E*)-tetradeca-5,7,9,11-tetraen-1-yl (*R*)-2-hydroxy-3-methylbutanoate, a new natural compound, for which the name praealtaester A is proposed.

The alcohol moiety of **1**, but with a different configuration of double bonds (5*E*,7*E*,11*Z*,13*E*), was reported as part of a not fully characterized ester isolated from two *Centaurea* species (Asteraceae)[18,19] and, later on, from *C. scabiosa*, an isovalerate, but this time it was isolated with an undetermined configuration of the double bonds.[20] Low amounts of **2**, an isovalerate of tetradeca-5,7,9,11-tetraen-1-ol, were also present in the *B. praealtum* extract. To elucidate the structure of **2**, a sample of the ester of isovaleric acid and (5*Z*,7*E*,9*E*,11*E*)-tetradeca-5,7,9,11-tetraen-1-ol, obtained by a careful methanolysis of **1**, was prepared in a Steglich-type reaction. A GC−MS coinjection experiment confirmed the existence of **2**, (5*Z*,7*E*,9*E*,11*E*)-tetradeca-5,7,9,11-tetraen-1-yl 3-methylbutanoate (praealtaester B), in *B. praealtum* aerial parts, but **2** appears to be absent from the ripe schizocarps.

An isomer of **1** (**3**) and an acetylated derivative of **1** (**4**, Figure 1) were also detected in the *B. praealtum* extract by GC−MS. The retention index (DB-5MS) of **3** was 38 units higher than that of **1** (RI(**3**) = 2403 and RI(**1**) = 2365), and their mass spectra were almost identical. These were indicative of a relationship of *cis*- and *trans*-geometric isomers, in this case corresponding to **1** and **3**, respectively, as exemplified by the values of retention indices of a trienic system (RI-(*trans*,*cis*,*trans*-isomer) = 1598 and RI(*trans*,*trans*,*trans*-isomer) = 1648).[21] This means that **3** differs from **1** only in the geometry of the C-5′−C-6′ double bond. An enriched sample of **3** (76%) was obtained from further fractionations, while 1D and 2D NMR spectra of this mixture (Figures S3.1, and S3.2, Supporting Information), along with the data from the previously simulated spectra of **1**, enabled a full spectral assignment (Tables 1 and 2) and unambiguously confirmed that **3** is indeed the "all-*trans*" isomer, i.e., (5*E*,7*E*,9*E*,11*E*)-tetradeca-5,7,9,11-tetraen-1-yl 2-hydroxy-3-methylbutanoate (praealtaester C, Figure 1). It is possible that **3** merely represents an artifact of **1**, a thermodynamically more stable isomer formed as a consequence of absorption of UV light.[19] This, on the other hand, may suggest a possible protective function of **1** and related compounds in the plant against harmful UV irradiation. This gradual conversion to an "all-*trans*" isomer was also actually noticed in the case of **2**, occurring spontaneously, and was most probably light-driven. The freshly synthesized **2** contained only small amounts (based on ^{1}H NMR, less than 2%) of all-*trans*-**2**, while after a week (Figure S2.3, Supporting Information), the molar percentage rose to 22%; however, the sample was stored in a closed vial at 4 °C in the dark.

A comparison of the mass spectra of **4** and **1**, i.e., the presence of a dominant m/z 43 and an increment in the mass of 42 in the M^+ ion, as well as their RI values, led to an assumption that **4** (Figure 1) is an acetylated derivative of **1**. This hypothesis was confirmed by a synthesis of the acetate of **1** and a GC−MS coinjection experiment. The prepared sample was utilized to acquire all of the pertaining analytical data for

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this new compound (praealtaester D, **4**) as well (Tables 1 and 2).

One more highly unsaturated, polyenic compound, **5** (Figure 1), was isolated from the *B. praealtum* aerial parts' extract and was found to have the molecular formula $C_{14}H_{18}O_2$ based on HRMS. The sharp and intense IR band at 1771 cm^{-1} straightforwardly revealed the presence of a γ-lactone moiety, accounting for two of the six total unsaturations.[22] The remaining four unsaturations were allocated to four conjugated double bonds which were initially inferred from the observed absorption maxima in the UV spectrum of **5** and corroborated by the expected eight methyne sp^2-hybridized carbon-atom signals in its ^{13}C NMR/DEPT-90 spectra. ^{13}C NMR and ^1H data (Tables 1 and 2) of **5** gave further evidence of a methyl group (a triplet at 1.02 ppm), three methylenes, one more methyne singly bonded to an oxygen atom, and a lactone carbon at 177.2 ppm. The signal at δ_H 5.00, corresponding to H-4, provided the connectivity data of the lactone with the rest of the molecule, via proton−proton coupling constants of 7.7, 7.0, and 6.8 Hz, and appropriate long-range C−H couplings (Figure S5.4, Supporting Information). Hence, while the lactone was on one side of the tetraenic system, the other was occupied by an ethyl group, as in the cases of **1**−**4**. The appropriate ^1H full spin spectral simulation and a concomitant comparison with the ^1H chemical shifts and coupling constant values of **1**−**4** permitted the assignment of the (all-*E*)-configuration of the four double bonds.

The absolute configuration of the chiral center at C-4 of **5** was deduced after a total hydrogenation experiment. The specific optical rotation of the obtained γ-tetradecalactone matched the sign and value of the angle of rotation of the (*R*)-enantiomer.[23] This means that the chiral center in **5** has the (*S*)-configuration due to the formal change in the Cahn−Ingold−Prelog priority of groups around the center upon hydrogenation. A related doubly unsaturated lactone having the (*S*)-configuration at C-4, synthesized in a stereoselective manner,[24] was also determined to have the same sign (+) of rotation as that of **5**. Herein, the new natural lactone **5** is concluded to be (*S*)-5-((1*E*,3*E*,5*E*,7*E*)-deca-1,3,5,7-tetraen-1-yl)dihydrofuran-2(3*H*)-one, for which the name praealtalactone is proposed.

Two related dibenzylbutyrolactones were additionally isolated from *B. praealtum* extracts, one of which is a known compound, from the *B. praealtum* aerial parts' extract, straightforwardly identified as (+)-arcitin (*syn. O,O*-dimethylmatairesinol, Figure S6.5, Supporting Information), by comparison of its NMR data with the previously reported ones.[25] The NMR, MS, and IR spectra of the other lignan (**6**, Figure 1), isolated from the *B. praealtum* schizocarp extract, pointed to an oxygenated derivative of arcitin, possessing a keto function in position 7. It appears that, in deuterated chloroform, **6** exists solely in the keto tautomeric form, as no traces of the enolic form (Figure S6.5, Supporting Information), expected for β-ketolactones, were visible in the NMR spectra. These enolic tautomers were neither mentioned in the synthetic work on closely related keto-lignans nor for the naturally occurring conicaol B.[26] The data from the mentioned papers were also helpful in establishing the relative configuration of the chiral centers at 8 and 8′, as the observed NOESY interactions of **6** were inconclusive: There were both cross-peaks noted between H-8 and H-8′ and H-8 and H-7′a/b. As in the structure of arcitin, the two substituted benzylic groups on the highly flexible γ-butyrolactone ring of **6** were found to be mutually *trans* based on the pertinent spectral data comparison.[26] In this way, **6** was recognized as 7-oxoarcitin, or *O*-methylconicaol B, a compound previously unknown from natural sources but reported almost 60 years ago as an insufficiently characterized (only UV and IR data provided) synthetic intermediate.[27] Thus, herein, for the first time, its full NMR assignment (Figures S6.1 and S6.2, Supporting Information) was performed, and it represents a new natural product.

Chromatography of the extract of *B. veronense* yielded compound **7** (Figure 2), with an EIMS similar to that of

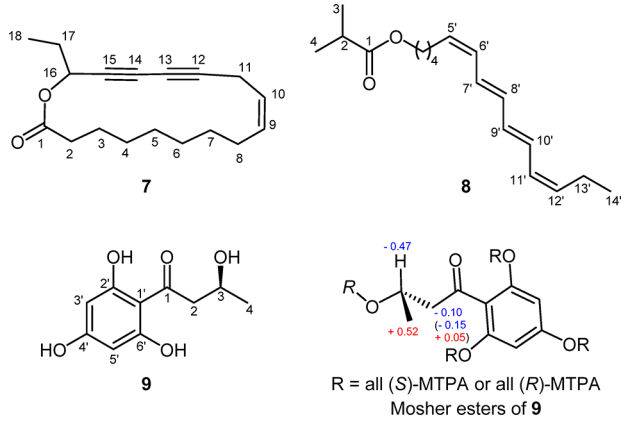

Figure 2. Structures of compounds **7**−**9** isolated from *B. veronense* and the Mosher esters of **9** with relevant ^1H NMR data.

falcarinol,[28] a dienediyne secondary alcohol. Its molecular ion corresponded to the formula $C_{18}H_{24}O_2$, established by HREIMS, indicating seven indices of hydrogen deficiency; thus, there was one carbon and one oxygen atom, as well as one degree of unsaturation more than that of falcarinol. The IR spectrum revealed the presence of a carbonyl (1727.5 cm^{-1}) group, suggesting the presence of an ester functionality. The 18 carbon signals were all resolved in the ^{13}C NMR spectrum (Table 3), which is in accordance with the molecular formula, and included a carbonyl (δ_C 173.5), a methyl (δ_C 9.6), nine sp^3 methylenes, one oxygenated sp^3 (δ_C 65.3), and two sp^2 methyne groups, as well as four sp nonprotonated carbons (δ_C 64.7, 70.4, 74.5, and 80.5). The aforementioned functionalities accounted for six degrees of unsaturation, indicating that **7** also contains one cycle. The ester carbonyl C-1 (δ_C 173.5) was shown to be attached to C-2 by the gHMBC correlations of H-2a and H-2b to C-1. The gradient $^1H-^1H$ COSY data established the presence of a long, proton-bearing and spin-coupled structural unit, vicinally coupled from C-2 to C-11 and from C-16 to C-18, while the two moieties are mutually connected via a long-range coupling constant over seven bonds of 0.7 Hz, i.e., over two conjugated triple bonds (Figure S7.4, Supporting Information). The analysis of gHMBC data of **8** corroborated the connectivity, whereas the ester/lactone group was positioned on the grounds of H-16−C-1 three-bond coupling, indicating that a rare 17-membered macrolide was formed (Figure S7.4, Supporting Information). The presence of a double bond, in the same position as in falcarinol, was evident initially from the chemical shifts of H-9 (δ_H 5.58, δ_C 135.2) and H-10 (δ_H 5.49, δ_C 121.4), and it was assigned (*Z*)-configuration based on the H-9−H-10 coupling constant value (J = 10.3 Hz) and confirmed by multiple observed gHMBC correlations of H-

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Table 3. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100.6 MHz, $CDCl_3$) NMR Spectroscopic Data of 7 and (*Z*)-Falcarinol

homodihydrofalcarinolide (7)			(*Z*)-falcarinol[46]		
position	δ_C, type	δ_H[a], (J[b] in Hz)	position	δ_C, type	δ_H, (J in Hz)
1	173.5, C	c	17		d
2a	34.7, CH_2	2.35, ddd (−13.3, 9.8, 4.1)	16	22.6, CH_2	1.22−1.31
2b		2.31, ddd (−13.3, 4.3, 7.5)			
3a	26.6, CH_2	1.70, m	15	31.9, CH_2	
3b		1.64, m			
4	28.80, CH_2	1.35[a], m	14	29.2, CH_2	overlapping peaks
5	29.8, CH_2	1.30[a], m			
6	30.3, CH_2	1.34[a], m	13		
7	28.77, CH_2	1.43, m 1.3820, m	12		1.37, m
8a	28.4, CH_2	2.11, ddddd (−13.8, 8.4, 7.0, 6.6, −1.2)	11	27.2, CH_2	2.02, q[e] (7.1)
8b		2.06, ddddd (−13.8, 7.6, 6.7, 6.5, −1.2)			
9	135.2, CH	5.58, dddt[e] (10.3, 8.4, 7.6, −1.1)	10	133.1, CH	5.52, dtt (10.5, 7.1, 1.5)
10	121.4, CH	5.45, dt[e]t[e] (10.3, −1.2, −1.1)	9	121.9, CH	5.37, dtt (10.5, 6.9, 1.5)
11a	17.0, CH_2	2.95, ddd (−17.9, 7.4, −1.1)	8	17.7, CH_2	3.03, d (6.9)
11b		2.89, ddd (−17.9, 7.3, −1.1)			
12	80.5, C	c	7	80.2, C	c
13	64.7, C	c	6	64.0, C	c
14	70.4, C	c	5	71.2, C	c
15	74.5, C	c	4	74.3, C	c
16	65.3, CH	5.28, t (6.7)	3	63.5, CH	4.92, d[e]quint (5.3, 1.1)
17	27.3, CH_2	1.79, dq (7.4, 6.7)	2		d
18	9.6, CH_3	1.00, t (7.4)	1		

[a]Chemical shift and coupling constant values were inferred from ^{1}H homoselective decoupling experiments and spin simulation. [b]$|J| < 0.7$ values are not listed for the sake of easier comparison. [c]No protons or no position. [d]Signals are not useful for comparison sake. [e]Pseudo, i.e., a pseudo multiplet. For example, a doublet of doublets but with two similar J values would likely appear as a pseudotriplet.

8−C-9 and C-10, H-10−C-9 and C-11, and H-11−C-10 (Figure S7.4, Supporting Information). Two- or three-bond couplings, noted in the gHMBC spectrum, of H-11 to C-12 (δ_C 80.5) and C-13 (δ_C 64.7) and of H-16 to C-14 (δ_C 70.4) and C-15 (δ_C 74.5) evidenced that a conjugated diyne moiety (C-12 to C-15, four sp-hybridized carbons) was bridging C-11 and C-16. Hence, compound **7** was concluded to be (*Z*)-17-ethyloxacycloheptadeca-10-ene-13,15-diyn-2-one, a new natural compound likely biosynthetically related to (*Z*)-falcarinol, for which the name homodihydrofalcarinolide is proposed.

The closest-related compound to **7** is an immunosuppressive macrolide ivorenolide B, isolated from the African mahogany *Khaya ivorensis* belonging to Meliaceae.[29] Ivorenolide B possesses two additional oxygens: Instead of the C-9−C-10 double bond, there is a *cis*-epoxide ring, and in the allylic-propargylic position C-11, an alcoholic group is located. It seems that (*Z*)-falcarinol and homodihydrofalcarinolide (**7**) share the same biosynthetic background; it is believed that a 17,18-didehydro acid corresponding to the nonlactonized **7** is the precursor that undergoes decarboxylation to falcarinol.[30] Compound **7** might represent either a missing link in the biosynthetic pathway from crepenynic acid to falcarinol derivatives or a diverging pathway.

Compound **8**, isolated from *B. veronense*, displayed in its MS, as observed for praealtaester A (**1**), the same dominant fragmentation ions (m/z 91, 105, 117, etc.), indicative of a high degree of unsaturation. The molecular ion (at m/z 276) suggested that **8** might be a related compound to **1**, an ester of a tetradecatetraenic alcohol and a saturated four-carbon carboxylic acid. As described above for **1**−**4**, the analyses of ^{1}H and ^{13}C NMR spectra led to the conclusion that **8** is indeed tetradeca-5,7,9,11-tetraen-1-yl 2-methylpropanoate (Figure 2). The appearance of olefin proton signals in the ^{1}H NMR of **8** immediately conveyed that the configuration(s) of the double bonds differed from those in both **1**−**2** and **4** (5*Z*,7*E*,9*E*,11*E*) and **3** (5*E*,7*E*,9*E*,11*E*). The geometry of the C-5′−C-6′ and C-11′−C-12′ double bonds in **8** were both assigned to be (*Z*) from the corresponding *cis*-proton−proton ($J_{H\text{-}5'\text{–}H\text{-}6'}$ = 11.2 and $J_{H\text{-}11'\text{–}H\text{-}12'}$ = 11.3 Hz) and *trans*-proton−carbon ($J_{H\text{-}6'\text{–}C\text{-}4'}$ = 12.0, $J_{H\text{-}5'\text{–}C\text{-}7'}$ = 11.3, and $J_{H\text{-}12'\text{–}C\text{-}10'}$ = 11.0 Hz) coupling constant values, either directly mined from the spectra or derived from an appropriate spectral simulation; the latter gave all other coupling constant values (Figure S1.6, Supporting Information). Similarly, the configurations of the two inner double bonds were both determined to be (*E*), as in **1**−**4**. The comparison of ^{13}C and ^{1}H NMR spectra of **8** with those of the corresponding methyl parinarate isomer[16] (Tables S1 and S2, Supporting Information) confirmed the (5*Z*,7*E*,11*E*,13*Z*)-configuration. Thus, (5*Z*,7*E*,11*E*,13*Z*)-tetradeca-5,7,9,11-tetraen-1-yl 2-methylpropanoate (**8**, in analogy to **1**−**4**, veronaester) is a new natural compound from *B. veronense*, related to the ones from *B. praealtum*, differing in the identity of the esterifying acids and the configuration of the tetraenic alcohol. Speaking in chemotaxonomical terms, their existence provides support for the infrageneric placement of both taxa within the same section of the genus.

Compound **9** (Figure 2), located in one of the most polar chromatographic fractions of the *B. veronense* extract, was subjected to silylation. A multiply trimethylsilylated derivative of **9** was obtained, whose MS, after the loss of a $C_7H_{14}O_2Si$ fragment, was highly similar to that of phloroglucinol-triTMS,[31] indicating the presence of three phenolic and one alcohol OH groups. Its IR spectrum contained an absorption band at 1630 cm^{-1}, suggesting the presence of an intramolecularly hydrogen-bonded carbonyl function conjugated with a highly electron-donating group, and a broad ∼3241

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Table 4. ^{1}H (400 MHz) and ^{13}C (100.6 MHz) NMR Data of Veroglucinol (9) in DMSO-d_6 and Methanol-d_4

	DMSO-d_6		methanol-d_4			
position	δ_C, type	δ_H, (J in Hz)	δ_C, type	δ_H, (J in Hz)	gHMBC[a]	NOESY[b]
1	203.6, C	[c]	205.2, C	[c]	[c]	[c]
2a	52.9, CH_2	3.13, dd (−15.8, 7.1)	53.9, CH_2	3.22, dd (−16.0, 5.3)	1, 3	4
2b		3.09, dd (−15.8, 5.6)		3.18, dd (−16.0, 7.5)		
3	63.4, CH	4.20, dqd (7.1, 6.1, 5.6)	65.7, CH	4.35, dqd (7.5, 6.1, 5.3)	2, 4	2, 4
4	23.8, CH_3	1.13, d (6.1)	23.7, CH_3	1.23, d (6.1)	2, 3	3
1′	104.3, C	[c]	104.3, C	[c]	[c]	[c]
2′, 6′	164.3, C	[c]	166.0, C	[c]	[c]	[c]
3′, 5′	94.8, CH	5.83, s	95.9, CH	5.81, s	1′, (5′, 3′)	[c]
4′	164.7, C	[c]	166.4, C	[c]	[c]	[c]
OH-2′, 6′	[c]	12.22, s	[c]	[c]	2′, 6′	[c]
OH-4′	[c]	10.32, s	[c]	[c]	4′	[c]

[a]Long-range coupling between the proton in the position column with the carbon in the HMBC column. [b]NOE observed between the proton in the position column with the proton in the NOESY column. [c]No protons or no position.

cm^{-1} band corresponding to several hydroxyl functions; the occurrence of a conjugated ketone was additionally confirmed by the ^{13}C NMR (DMSO-d_6) signal noted at 203.6 ppm (Table 4). The number, integrals, and singlet nature of the ^{1}H NMR signals at 12.22 ppm (OH-2′ and 6′) and 10.32 (OH-4′), corresponding to exchangeable phenolic hydrogens, and 5.83 ppm (H-3′ and H-5′), corresponding to protons on an electron-rich aromatic ring, indicate that the core in **9** is symmetrically substituted. The symmetry in the aromatic ring was also evident from the *meta* coupling (2.3 Hz) of the chemically equivalent H-3′ and H-5′ visible in the splitting of ^{13}C-satellites in its ^{1}H NMR (Figures S9.1 and S9.3, Supporting Information). Chemical shifts of diastereotopic H-2a (3.13 ppm) and H-2b (3.09 ppm), along with their gHMBC interactions with C-1 and C-3 (Figure S9.7, Supporting Information), positioned them α to the carbonyl and suggested the presence of a secondary alcohol chiral center in an aldol-type moiety (C-3, 63.4 ppm). It was concluded that **9** is 3-hydroxy-1-(2,4,6-trihydroxyphenyl)butan-1-one, a relatively simple acylphloroglucinol derivative and a new natural product (Figure 2).

The determination of the configuration of the chiral center at C-3 required preparation of diastereomeric Mosher esters using the commercially available enantiopure acids. The ^{1}H NMR data of the prepared esters permitted us to assign the configuration (*S*) to the center (Figure 2), which was additionally confirmed by comparing the relevant ^{1}H NMR chemical shifts with literature values for related compounds obtained in an enantiospecific Baker's yeast reduction step.[32] The name veroglucinol is proposed for **9**, (*S*)-3-hydroxy-1-(2,4,6-trihydroxyphenyl)butan-1-one, due to its very restricted occurrence in nature.

Rechromatography of the fraction preceding the one containing **9** yielded two additional compounds, a dehydrated and a cyclized (**9a** and **9b**, respectively, Figure S9.6, Supporting Information) derivative of **9**. Compound **9a** is mentioned in the literature only as a synthetic intermediate[33] and has never been isolated from a natural source to date. The cyclized derivative **9b** was previously isolated from a plant species *Aphanamixis grandifolia*[34] and a fungus *Xylaria nigripes*.[35] It appears that the three compounds, **9**, **9a**, and **9b**, might be either biosynthetically or artifactually interconnected; because **9** exists as a single enantiomer, it seems that a (facile) dehydration of the β-hydroxyketone, giving a conjugated enone (**9a**), and a subsequent intramolecular Michael addition, yielding a chromanone derivative (**9b**), is the most likely course of these transformations. After we keep in mind that only traces of **9a** and **9b** were isolated and that they were practically coeluting with **9**, more arguments lay in favor of the artifactual nature of the two additional phloroglucinols.

Two more lignans were isolated from the extract of *B. veronense*, isoyatein,[36] and lariciresinol[37] (Figure S6.5, Supporting Information). Isoyatein is a rare dibenzylbutyrolactone derivative previously isolated from *Piper cubeba*[36] and detected in *Callitris preissii*.[37] Lariciresinol is widely occurring in plants[38] but has not been previously known to be a secondary metabolite of *Bupleurum* spp.

Compounds **1**, **4**, **5**, and (+)-arcitin, isolated in sufficient quantity, along with cisplatin (0.1 mM), were assessed for their effects in the concentration range 10^{-5}−10^{-1} mM on splenocyte (SPC) and macrophage (Mφ) viability and functional characteristics. All compounds altered, in a statistically significant manner (increased and/or decreased), mitochondrial and lysosomal functions of SPCs and Mφs, based on their ability to metabolize MTT (Figure 3) and accumulate NR (Figure S10, Supporting Information). Compounds **1**, **4**, and **5** share a common structural motif, a deca-1,3,5,7-tetraen-1-yl chain, which is also a characteristic of parinaric acids (Figure S10, Supporting Information). The latter acids were established to affect the viability of human leukemia cells (THP-1 monocytic leukemia and HL-60 human promyelocytic leukemia) at concentrations of 5 μM or less.[39] In comparison to the cytotoxic effect of parinaric acids, praealtalactone (**5**) significantly reduced the viability of both SPCs and Mφs in vitro only at 100 μM. However, both **1** and **4** in all tested concentrations and the <10 μM concentrations of **5** induced an opposite effect, potentiating the mitochondrial reductase ability, as well as their lysosomal function. This selective transformed-to-normal cell cytotoxicity was previously also demonstrated for α-parinaric acid in the case of nontumorous astrocytes.[40] Parinaric acids are believed to increase free radical production and, thus, sensitize transformed cells to lipid peroxidation, by means of the enhanced take-up of the acids by the rapidly growing malignant cells.[39,41]

The treatment of SPCs with (+)-arcitin (100 μM) decreased their NR-accumulating ability (Figure S10, Supporting Information); however, this effect was not found to be significant. Interestingly, when Mφs were exposed to the two highest concentrations of arcitin (100 and 10 μM, Figure S10, Supporting Information), the opposite effect to the one on

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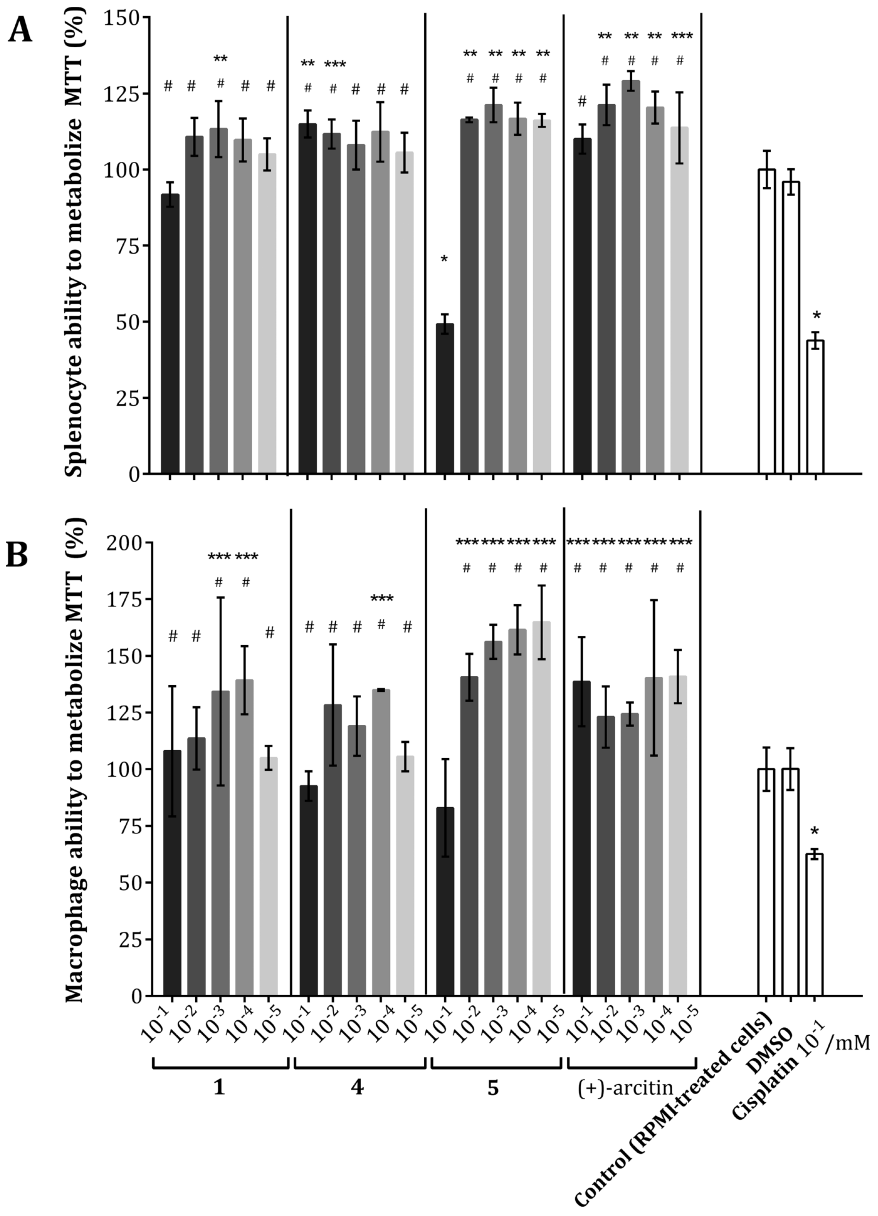

Figure 3. Effects of **1**, **4**, **5**, and (+)-arcitin on the abilities of splenocytes (A) and macrophages (B) to metabolize MTT. Data shown are the mean percentages (%) and SDs calculated based on the values obtained from RPMI-treated cells. Mutual comparison of the data was performed using one-way ANOVA, followed by Tukey's posthoc test: $^{*}p < 0.001$, $^{**}p < 0.01$, and $^{***}p < 0.05$ vs RPMI-treated cells; $^{\#}p < 0.001$ vs cisplatin treatment.

SPCs was found, i.e., a statistically significant increase in Mφ ability to accumulate NR (Figure S10, Supporting Information). Arcitin was previously found to exhibit marked cytotoxicity toward Jurkat T cells (immortalized human T lymphocytes)[25] and to lower the amount of IgE in cultures of U266 cells in a concentration-dependent manner (immortalized human B lymphocytes).[42] The present results with primary cell cultures of rat spleen lymphocytes (and macrophages) showed that (+)-arcitin does not reduce their viability but stimulates their ability to both metabolize MTT and accumulate NR, suggesting a functional potentiation of these immune cells. Thus, it appears that this lignan exerts a selective cytotoxic effect toward transformed lymphocytes, while it does not affect the viability and/or function of normal cells.

Praealtalactone (**5**), predominantly, and praealtaester A (**1**), to a lesser extent, showed potential in altering the activity of Mφ myeloperoxidase (Figure 4). This activity can be related either to their ability to inhibit MPO or to their potential to react with free radicals[43] generated by MPO, suggesting their direct involvement in the oxidative cell processes caused by reactive oxygen species similar to the α-parinaric acid.[39] This antioxidant nature of **1**, **4**, and **5**, in cell culture, suggests a possible protective role in plant tissues. The more pronounced cytotoxic nature of **5** could be explained by its potentially different metabolism in cells, leading potentially to the formation of Michael acceptor-type cytotoxic agents. It is known that aliphatic γ-lactones undergo hydrolysis catalyzed by paroxonidases, followed by steps of α- or β-oxidative transformation of fatty acids.[44] In the case of **5**, after hydrolysis and initial oxidation of the newly formed alcohol group, the resulting α,β-unsaturated conjugated ketone appears to be the likely Michael acceptor, i.e., a possible alkylating agent. One

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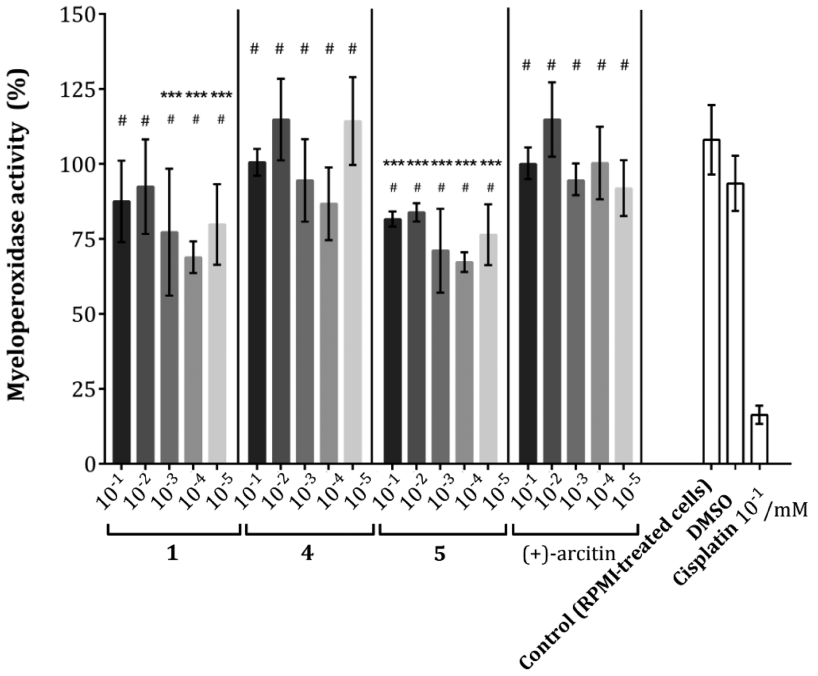

Figure 4. Myeloperoxidase (MPO) activities in macrophages (Mφs) treated with **1**, **4**, **5**, and (+)-arcitin. Data shown are the mean percentages (%) and SD calculated based on the values obtained from RPMI-treated cells. Mutual comparison of the data was performed using one-way ANOVA, followed by Tukey's posthoc test: $^{*}p < 0.001$, $^{**}p < 0.01$, and $^{***}p < 0.05$ vs RPMI-treated cells; $^{\#}p < 0.001$ vs cisplatin treatment.

can conclude that the enthopharmacologically praised *Bupleurum* taxa warrant their status of immunomodulatory-acting herbal medicines, more specifically, such herbal medicines that boost the functional characteristics of normal SPCs and Mφs, although specific metabolites might have a different effect on their own.

EXPERIMENTAL SECTION

General Experimental Procedures. All used chemicals and solvents were obtained from commercial sources (Sigma-Aldrich, St. Louis, MO, USA; Merck, Darmstadt, Germany; Fisher Scientific, Waltham, MA, USA) and used as received, except for the solvents, which were predistilled and dried before use. Silica gel used for preparative separations (both dry-flash and column) was silica gel 60 (0.04−0.063 mm, Merck, Darmstadt, Germany), while gel filtrations were done on Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Optical rotations were measured in MeOH at 25 °C using an Autopol IV (Rudolph Research Analytical, Hackettstown, New Jersey, USA) polarimeter equipped with a sodium lamp (589 nm) and a 10 cm microcell. UV measurements were carried out on a Shimadzu UV-1800 UV−vis spectrophotometer (Kyoto, Japan). IR spectra (ATR-attenuated total reflectance) were recorded on a Thermo Nicolet model 6700 FT-IR instrument (Waltham, MA, USA). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz NMR spectrometer (Fällanden, Switzerland; ^{1}H at 400 MHz, ^{13}C at 100.6 MHz), equipped with a 5 mm dual ^{13}C/^{1}H probe head at 20 °C. All the NMR spectra were recorded in chloroform-*d*, methanol-d_4, or DMSO-d_6 (Sigma-Aldrich, St. Louis, MO, USA) with tetramethylsilane as the internal standard. Chemical shifts (δ) are reported in ppm and referenced to tetramethylsilane (δ_H = 0.00 ppm), or the (residual) solvent signal ($CHCl_3$, CHD_2OD, CHD_2SOCD_3), and $^{13}CDCl_3$, $^{13}CD_3OD$, and $^{13}CD_3SOCD_3$, in ^{1}H NMR and ^{13}C NMR and heteronuclear 2D spectra, respectively. Scalar couplings are reported in Hertz (Hz). The acquired NMR experiments, both 1D and 2D, were recorded using standard Bruker built-in pulse sequences. GC−MS analyses (three repetitions) were carried out using a Hewlett−Packard 6890N gas chromatograph equipped with a fused silica capillary column DB-5MS ((5%-phenyl)-methylpolysiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, Santa Clara, California, USA) and coupled with a 5975B mass selective detector of the same company. Retention indices (RIs) were determined using a mixture of *n*-alkanes from C_8−C_{40}. Analytical TLC separations were done on coated Al silica gel plates (Kieselgel 60 F_{254}, 0.2 mm, Merck, Darmstadt, Germany). TLC plates were initially visualized by UV light (254 nm) and then sprayed with 50% (w/w) aqueous H_2SO_4 followed by heating. High-resolution mass spectrometry (HRMS) analysis was performed using an MStation JMS-700 mass spectrometer (JEOL, Peabody, MA, USA) with an ionization energy of 70 eV, an ionization trap current of 300 μA, and a source temperature of 230 °C.

^{1}H NMR full spin analysis of compounds **1**−**4** and **7**−**9** was performed by manually adjusting δ_H and *J* values to fit the experimentally available values and further optimized using MestReNova 11.0.3 software (tools/spin simulation). This procedure led to a systematic refinement of all calculated NMR parameters until the simulation outcome was in excellent agreement (NRMSD < 0.05%) with the experimental data of the isolated compounds.

Plant Material. The above-ground plant parts of *Bupleurum praealtum* in the intermediate flowering-fruit-bearing phase were collected in August 2015 on the slopes of Suva Planina Mt. (near Niš, southeastern Serbia, 43°11′53.1″N 22°08′33.6″E), while the aerial parts of *B. veronense*, fully flowering, were collected in June 2018 on Pelješac Peninsula (near Mali Ston, Dalmatia, Croatia, 42°50′39.7″N 17°42′11.8″E), both from single populations. Voucher specimens have been deposited in the Herbarium of the Faculty of Sciences and Mathematics, University of Niš (voucher nos. HMN 12112 and HMN 13669 for *B. praealtum* and *B. veronense*, respectively). The plant material was identified by one of the authors (V.R.).

Extraction and Isolation. Before air-drying to constant mass, the umbels of *B. praealtum* were carefully detached from the rest of the aerial parts; the dry umbels were lightly shaken to free the ripe schizocarps which were additionally sieved. The aerial parts of *B. praealtum* were cut into small pieces, whereas the *B. praealtum* schizocarps were mechanically ground and extracted separately by maceration with Et_2O for 10 days with occasional shaking at room temperature protected from light. The dried plant material of *B. veronense* was treated in an analogous manner, except the entire aerial

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parts were extracted together. The extracts were dried with anhydrous $MgSO_4$ and gravity filtered to remove all insoluble material before the removal of Et_2O at room temperature in vacuo. The yields of the *B. praealtum* aerial parts, *B. praealtum* schizocarp, and *B. veronense* Et_2O extracts were 2.3, 1.9, and 2.5% (w/w, based on dry plant mass), respectively.

All of the extracts were subjected to silica gel dry-flash chromatography under gradient conditions using mixtures of increasing polarity starting with hexane−Et_2O, through Et_2O−EtOAc, and ending in EtOAc−methanol mixtures. Fraction 19 (hexane−Et_2O = 2:1, v/v, 97 mg) of the *B. praealtum* schizocarp extract represented pure compound **1**, while fraction 18 (hexane−Et_2O = 2:1, v/v) was a mixture mostly composed of **1**, **3**, and **4**; fraction 25 eluted with the mixture of Et_2O−EtOAc (1:1, v/v, 20 mg) was pure compound **6**. Because fractions 18 and 19 of the *B. praealtum* aerial parts' extract (hexane−Et_2O = 2:1, v/v) represented a similar mixture of **1**, **3**, and **4**, these fractions and fraction 18 from the *B. praealtum* schizocarp extract were pooled and subjected to further separation, first on Sephadex LH-20 (isocratic, $CHCl_3$−MeOH = 1:1, v/v) and then by silica gel-column chromatography (hexane−Et_2O = 2:1, v/v). These resulted in additional quantities of pure **1** and a mixture of **1** and **3** considerably more enriched in **3**, while **4** remained in a mixture. Compound **5** (23 mg) was isolated from the *B. praealtum* aerial parts' extract in the first silica gel dry-flash chromatography, located in fraction 25 which was eluted with hexane-Et_2O (1:3, v/v). Fraction 30 (EtOAc−MeOH, 7:3, v/v), 12 mg, represented pure (+)-arcitin (synonym dimethylmatairesinol).[25] Additionally, the initial chromatography of the *B. praealtum* aerial parts' extract resulted in fractions 11 and 12, 5, and 10 mg, respectively, both eluted with 15% (v/v) Et_2O in hexane, that contained, based on GC−MS analyses, compound **2**, unfortunately in a complex mixture. Its identity was hinted from its mass spectrum and corroborated by synthesis, as detailed below.

*(5Z,7E,9E,11E)-Tetradeca-5,7,9,11-tetraen-1-yl (R)-2-Hydroxy-3-methylbutanoate (Praealtaester A, **1**).* This compound is a yellowish oil. $[\alpha]^{25}_D$ + 64.3 (*c* 1, MeOH). UV (CH_3CN) λ_{max} (log ε) 289 (4.28), 302 (4.54), 316 (4.51), 341 (2.95) nm. IR (neat) ν_{max} ~ 3500, 2961, 2916, 2848, 1727, 1462, 1138, 996 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) data, see Tables 2 and 1. EIMS *m/z*: $[M]^+$ 306 (99), 159 (42), 131 (58), 119 (36), 117 (70), 105 (49), 91 (100), 79 (51), 73 (34), 55 (39). HREIMS *m/z*: $[M]^+$ 306.4459 (calcd for $C_{19}H_{30}O_3$, 306.4460). RI (DB-5MS) 2365.

*(5Z,7E,9E,11E)-Tetradeca-5,7,9,11-tetraen-1-yl 3-Methylbutanoate (Praealtaester B, **2**).* This compound is a colorless oil. ^1H NMR ($CDCl_3$, 400 MHz): δ 6.44 (1H, dddd, *J* = 14.5, 11.3, 1.0, 0.7 Hz, H-7′), 6.224 (1H, ddt, *J* = 14.5, 11.2, 0.7 Hz, H-8′), 6.217 (1H, ddd, *J* = 15.5, 10.8, 0.7 Hz, H-10′), 6.18 (1H, ddt, *J* = 15.5, 11.2, 0.7 Hz, H-9′), 6.09 (1H, ddtd, *J* = 15.0, 10.8, 1.4, 0.7 Hz, H-11′), 6.05 (1H, dddd, *J* = 11.3, 10.3, 1.4, 0.7 Hz, H-6′), 5.76 (1H, dt, *J* = 15.0, 6.8 Hz, H-12′), 5.38 (1H, dtd, *J* = 10.3, 7.4, 1.0 Hz, H-5′), 4.07 (2H, t, *J* = 6.6 Hz, H-1′), 2.33 (2H, d, *J* = 6.7 Hz, H-2), 2.24 (2H, tdd, *J* = 7.6, 7.4, 1.4 Hz, H-4′), 2.15 (2H, qdd, *J* = 7.5, 6.8, 1.4 Hz, H-13′), 2.08 (1H, t sept, *J* = 6.7, 6.6 Hz, H-3), 1.64 (2H, tt, *J* = 7.6, 6.6 Hz, H-2′), 1.46 (2H, pseudo quint, *J* = 7.6, 7.6 Hz, H-3′), 1.01 (3H, t, *J* = 7.5 Hz, H-14′), 0.95 (6H, d, *J* = 6.6 Hz, H-4 and H-5). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 173.5 (C, C-1), 137.4 (CH, C-12′), 133.5 (CH, C-10′), 133.3 (CH, C-8′), 131.6 (CH, C-5′), 130.9 (CH, C-9′), 129.6 (CH, C-11′), 129.4 (CH, C-6′), 127.4 (CH, C-7′), 64.2 (CH_2, C-1′), 43.6 (CH_2, C-2), 28.4 (CH_2, C-2′), 27.6 (CH_2, C-4′), 26.1 (CH, C-3), 26.0 (CH_2, C-3′), 25.8 (CH_2, C-13′), 22.6 (CH_3, C-4 and C-5), 13.7 (CH_3, C-14′). EIMS *m/z*: $[M]^+$ 290 (58), 131 (53), 117 (63), 105 (45), 91 (100), 85 (55), 79 (50), 67 (34), 57 (59), 41 (52). HREIMS *m/z*: $[M]^+$ 290.2250 (calcd for $C_{19}H_{30}O_2$, 290.2246). RI (DB-5MS) 2227.

*(5E,7E,9E,11E)-Tetradeca-5,7,9,11-tetraen-1-yl (R)-2-Hydroxy-3-methylbutanoate (Praealtaester C, **3**).* This compound is a colorless oil. For ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) data, see Tables 2 and 1. EIMS *m/z* $[M]^+$ 306 (52), 131 (47), 117 (62), 105 (42), 91 (100), 79 (51), 73 (40), 67 (34), 55 (43), 41 (37). HREIMS *m/z* 306.4456 $[M]^+$ (calcd for $C_{19}H_{30}O_3$, 306.4460). RI (DB-5MS) 2403.

*(5Z,7E,9E,11E)-Tetradeca-5,7,9,11-tetraen-1-yl (R)-2-Acetoxy-3-methylbutanoate (Praealtaester D, **4**).* This compound is a yellowish oil. $[\alpha]^{25}_D$ + 80.0 (*c* 1, MeOH). UV (CH_3CN) λ_{max} (log ε) 288 (4.81), 301 (4.97), 316 (4.92), 342 (3.84) nm. IR (neat) ν_{max} ~ 3500, 2961, 2916, 2848, 1727, 1462, 1138, 996 cm^{-1}. For ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) data, see Tables 2 and 1, respectively. EIMS *m/z*: $[M]^+$ 348 (21), 131 (17), 117 (26), 115 (52), 105 (19), 91 (37), 79 (19), 55 (14), 43 (100), 41 (22). HREIMS *m/z*: $[M]^+$ 348.4829 (calcd for $C_{21}H_{32}O_4$, 348.4830). RI (DB-5MS) 2485.

*(S)-5-((1E,3E,5E,7E)-Deca-1,3,5,7-tetraen-1-yl)dihydrofuran-2(3H)-one (Praealtalactone, **5**).* This compound is a colorless oil. $[\alpha]^{25}_D$ + 70.1 (*c* 1, MeOH). UV (CH_3CN) λ_{max} (log ε) 288 (2.64), 301 (2.80), 316 (2.74), 342 (2.07), 410 (2.64) nm. IR (neat) ν_{max} 2923, 2853, 2360, 2342, 1771, 1169, 1053, 1007 cm^{-1}. For ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) data, see Tables 2 and 1. EIMS *m/z*: $[M]^+$ 218 (30), 117 (34), 105 (52), 91 (100), 79 (65), 77 (56), 66 (38), 65 (34), 55 (37), 41 (58), 39 (56). HREIMS *m/z*: $[M]^+$ 218.2957 (calcd for $C_{14}H_{18}O_2$, 218.2960). RI (DB-5MS) 2107.

*7-Oxoarcitin ((3S,4R)-3-(3,4-Dimethoxybenzoyl)-4-(3,4-dimethoxybenzyl)dihydrofuran-2(3H)-one O-Methylconicaol B, **6**).* This compound is an amorphous solid. UV (MeOH) λ_{max} (log ε) 231 (4.08), 279 (3.90), 313 (3.69), 398 (3.41), 419 (3.42), 442 (3.42), 470 (3.32) nm. IR (neat) ν_{max} 2924, 2853, 1732, 1021 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 7.46 (1H, d, *J* = 2.1 Hz, H-2), 7.34 (1H, dd, *J* = 8.5, 2.1 Hz, H-6), 6.84 (1H, d, *J* = 8.5, H-5), 6.76 (1H, d, *J* = 8.1, H-5′), 6.69 (1H, dd, *J* = 8.1, 2.1 Hz, H-6′), 6.63 (1H, d, *J* = 2.1 Hz, H-2′), 4.54 (1H, dd, *J* = 9.0, 7.0, H-9′a), 4.27 (1H, d, *J* = 6.0, H-8), 4.15 (1H, dd, *J* = 9.0, 5.7, H-9′b), 3.95 (1H, s, OMe-4), 3.91 (1H, s, OMe-3), 3.85 (1H, s, OMe-4′), 3.80 (1H, s, OMe-3′), 3.41 (1H, m, H-8′), 2.84 (1H, dd, *J* = 13.7, 7.7, H-7′a), 2.78 (1H, dd, *J* = 13.7, 8.0, H-7′b). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 191.5 (C, C-7), 173.1 (C, C-9), 154.3 (C, C-4), 149.3 (C, C-3′), 149.3 (C, C-3), 148.2 (C, C-4′), 130.0 (C, C-1′), 128.8 (C, C-1), 124.7 (C, C-6), 121.2 (C, C-6′), 112.0 (C, C-2′), 111.4 (C, C-5′), 110.9 (C, C-2), 110.1 (C, C-5), 72.1 (CH_2, C-9′), 56.3 (CH_3, OMe-4), 56.1 (CH_3, OMe-3), 56.0 (CH_3, OMe-4′), 55.9 (CH_3, OMe-3′), 53.7 (CH, C-8), 41.4 (CH, C-8′), 38.0 (CH_2, C-7′). EIMS *m/z*: $[M]^+$ 400 (17), 178 (12), 177 (100), 165 (41), 151 (19), 146 (7), 137 (7), 107 (9), 79 (7), 77 (8). HREIMS *m/z*: $[M]^+$ 400.4272 (calcd for $C_{22}H_{24}O_7$, 400.4270). RI (DB-5MS) 3310.

Fractions 11 and 12 of the *B. veronense* extract (hexane−Et_2O = 6:1, v/v, 99 mg in total) were subsequently isocratically (hexane−Et_2O = 7:1, v/v) chromatographed on a longer silica gel column giving pure **7** (19 mg) and **8** (5 mg). Fraction 25 (EtOAc−MeOH = 9:1, v/v) of the original dry-flash chromatography was pure **9** (58 mg), while fraction 24, after being rechromatographed on Sephadex LH-20 (isocratic, $CHCl_3$−MeOH = 1:1, v/v), yielded pure minute samples (less than a milligram) of **9a**[33] and **9b**,[33] as well as the lignans isoyatein (11 mg),[36] and lariciresinol (3 mg).[37]

*(Z)-17-Ethyloxacycloheptadeca-10-ene-13,15-diyn-2-one (Homodihydrofalcarinolide, **7**).* This compound is a transparent oil. UV (CH_3CN) λ_{max} (log ε) 216 (3.11), 252 (2.86), 266 (2.83), 281 (2.74) nm. IR (neat) ν_{max} 2930, 2858, 1728 cm^{-1}. For ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) data, see Table 3. EIMS *m/z*: $[M]^+$ 272 (100), 129 (62), 128 (78), 117 (38), 115 (67), 105 (38), 91 (79), 77 (57), 55 (53), 41 (54). HREIMS *m/z*: $[M]^+$ 272.3877 (calcd for $C_{18}H_{24}O_2$, 272.3880). RI (DB-5MS) 2185.

*(5Z,7E,9E,11Z)-Tetradeca-5,7,9,11-tetraen-1-yl Isobutyrate (Veronaester, **8**).* This compound is a transparent oil. For ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) data, see Tables 2 and 1. EIMS *m/z*: $[M]^+$ 276 (59), 131 (58), 145 (35), 117 (69), 105 (46), 91 (100), 79 (48), 71 (45), 43 (66), 41 (38). HREIMS *m/z*: $[M]^+$ 276.4198 (calcd for $C_{18}H_{28}O_2$, 276.4200). RI (DB-5MS) 2127.

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*3-Hydroxy-1-(2,4,6-trihydroxyphenyl)butan-1-one (Veroglucinol, **9**).* This compound is a colorless oil. UV (MeOH) λ_{max} (log ε) 225 (4.25), 288 (4.35), 331 (3.59) shoulder nm. IR (neat) ν_{max} 3311, 2943, 2832, 1021 cm^{-1}. For ^1H NMR (DMSO-d_6 and methanol-d_4, 400 MHz) and ^{13}C NMR (DMSO-d_6 and methanol-d_4, 100.6 MHz) data, see Table 4.

Conversion of 1 to 4. To verify the structure of **4**, the compound was obtained by conversion of **1**. A solution of **1** (16 mg, 0.05 mmol), acetic acid (9 mg, 0.15 mmol), 4-(dimethylamino)pyridine (DMAP, 4 mg, 0.03 mmol), and *N*,*N*′-dicyclohexylcarbodiimide (DCC, 31 mg, 0.15 mmol) in 1 mL of dry CH_2Cl_2 was stirred in a vial overnight at room temperature, under argon. Afterward, the solvent was removed in vacuo; then, 3 mL of cold pentane was added to the residue, and the precipitated *N*,*N*′-dicyclohexylurea was filtered off. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography giving 7 mg (40% yield) of **4**.

Methanolysis of 1 and Synthesis of 2. A solution of **1** (18 mg) in methanol (1 mL) was added at room temperature to an excess of freshly prepared solution of sodium methoxide (ca. 1 g of Na in an excess of MeOH), protected from sunlight. The mixture was stirred and heated until reflux and then immediately cooled in ice water. Methanol was removed under reduced pressure at 30 °C, while the residue was partitioned between Et_2O and a saturated solution of sodium bicarbonate. The ether layer was washed several times with brine, and after the evaporation of Et_2O, (5*Z*,7*E*,9*E*,11*E*)-tetradeca-5,7,9,11-tetraen-1-ol was obtained and was used as is in the following step. Compound **2** was prepared starting from (5*Z*,7*E*,9*E*,11*E*)-tetradeca-5,7,9,11-tetraen-1-ol (3 mg, 0.01 mmol), isovaleric acid (3 mg, 0.03 mmol), DMAP (1 mg, 0.01 mmol), and DCC (6.2 mg, 0.03 mmol). The crude ester **2** was purified by silica gel column chromatography, and this gave 3 mg (71% yield) of pure **2**.

Hydrogenation of 6. A sample of **6** (10 mg) dissolved in EtOAc was mixed with 5 mg of Pd (10%) on charcoal, with the vigorously stirred reaction mixture flushed two times with hydrogen gas and kept under 1 atm of hydrogen for 2 h at room temperature. Afterward, the EtOAc solution was filtered through a small column of Celite, and the catalyst and the column were washed with EtOAc. EtOAc was removed from the pooled solutions in vacuo, and the residue was subjected to GC−MS to confirm that the hydrogenation was complete and that the γ-tetradecalactone obtained was pure (10 mg, 96%).

(R)-γ-Tetradecalactone. $[\alpha]^{20}_D$ + 30.1 (*c* 1, CH_3Cl), literature value $[\alpha]^{20}_D$ + 32 (*c* 1.1, $CHCl_3$).[23]

Synthesis of (+)- and (−)-Mosher's Acid Chlorides. Oxalyl chloride (131 μL, 1.5 mmol) was added to a solution of (*R*)-(+)- or (*S*)-(−)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA, 70 mg, 0.3 mmol) and DMF (25 μL, 0.3 mmol) in hexane (2 mL) at room temperature, under argon. After 1 h, the mixture was concentrated under a vacuum, and the resulting residue was used as such in the esterification step.

Preparation of (*R*)- and (*S*)-1a. Starting from 1.93 g of DL-valine and 1.00 g of L-valine (*R*/*S*- and *S*-, respectively), the corresponding (*R*/*S*)- and (*S*)-2-hydroxy-3-methylbutanoic acids were separately prepared, as described previously.[45] The crude acids were converted into methyl esters by the reaction with diazomethane and subjected to gradient column silica gel chromatography (0−100% Et_2O in hexane). In this way, pure methyl (*S*)-2-hydroxy-3-methylbutanoate, (*S*)-**1a**, was obtained in 20% yield (216 mg), while the racemic mixture of (*R*/*S*)-2-hydroxy-3-methylbutanoate, (*R*)- and (*S*)-**1a**, was isolated in 16% yield (182 mg). The purity of the esters was checked by TLC and GC−MS.

Preparation of (*R*)-MTPA Esters of (*R*)- and (*S*)-1a. (*R*)-MTPA and the prepared (*R*)- and (*S*)-**1a** were subjected to Steglich-type esterification, as described for **4**. The DCC-driven esterification was effectuated in 79% yield (28 mg) in the case of (*R*)-MTPA ester of (*S*)-**1a**, while the mixture of esters of (*R*)-MTPA and (*R*)- and (*S*)-**1a** was further separated by gradient silica gel chromatography (0−40%, v/v, Et_2O in hexane), to afford a pure sample of (*R*)-MTPA ester of (*R*)-**1a** (30 mg, 85%).

*(R)-MTPA Ester of (R)-**1a**.* This compound is a yellowish oil. For ^1H NMR ($CDCl_3$, 400 MHz) data, see Table S3 of the Supporting Information. EIMS *m*/*z*: $[M]^+$ 348 (1), 190 (44), 189 (97), 170 (47), 126 (31), 119 (17), 105 (36), 83 (100), 77 (23), 55 (49), 41 (17). RI (DB-5MS) 1701.

*(R)-MTPA Ester of (S)-**1a**.* This compound is a yellowish oil. For ^1H NMR ($CDCl_3$, 400 MHz) data, see Table S3 of the Supporting Information. EIMS *m*/*z*: $[M]^+$ 348 (1), 190 (44), 189 (97), 170 (47), 126 (31), 119 (17), 105 (36), 83 (100), 77 (23), 55 (49), 41 (17). RI (DB-5MS) 1718.

Preparation of (*R*)-MTPA Ester of 1. Under argon, a solution of **1** (23 mg, 0.07 mmol), Et_3N (28 μL, 0.35 mmol), and DMAP (a small crystal, ca. 1 mg) in $CHCl_3$ (2 mL) was added to the previously prepared crude chloride (*R*)-MTPA (35 mg, ca. 0.15 mmol). After 72 h of stirring, 10 mL of $CHCl_3$ were added, and the resulting solution was washed with brine (3 × 10 mL). The organic layer was dried with anhydrous $MgSO_4$ and concentrated in vacuo. After column chromatography on silica gel (10% of Et_2O in hexane, v/v), pure (*R*)-MTPA ester of **1** was isolated in 31% yield (12 mg).

*(R)-MTPA Ester of **1**.* This compound is a yellowish oil. For ^1H NMR ($CDCl_3$, 400 MHz) data, see Table S3 of the Supporting Information. EIMS *m*/*z*: $[M]^+$ 522 (4), 189 (96), 186 (20), 159 (18), 145 (49), 133 (25), 131 (34), 119 (16), 117 (30), 105 (29), 91 (100). RI (DB-5MS) 2968.

Preparation of (*R*)- and (*S*)-MTPA Esters of 9. The previously prepared crude (*R*)- or (*S*)-MTPA chloride (ca. 15 mg, 0.06 mmol) was treated with **9** (7 mg, 0.03 mmol) in a similar manner to that described for **1**. Pure MTPA esters (14 mg (40%) and 12 mg (35%) for (*R*) and (*S*), respectively) were obtained by silica gel column chromatography (25% of Et_2O in hexane, v/v).

*(R)-MTPA Ester of **9**.* ^1H NMR ($CDCl_3$, 400 MHz): δ 5.93 (s, H-3′ and H-5′), 5.26 (1H, ddd, *J* = 7.0, 6.3, 5.5 Hz, H-3), 2.94 (1H, dd, *J* = −18.6, 5.5 Hz, H-2a), 2.74 (1H, dd, *J* = −18.6, 7.0 Hz, H-2b), 1.09 (3H, d, *J* = 6.3 Hz, H-4). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 195.5 (C, C-1), 164.3 (C, C-4′), 163.6 (C, C-2′ and C-6′), 104.6 (C, C-1′), 95.7 (CH, C-3′ and C-5′), 67.0 (CH, C-3), 49.0 (CH_2, C-2), 16.9 (CH_3, C-4).

*(S)-MTPA Ester of **9**.* ^1H NMR ($CDCl_3$, 400 MHz): δ 6.02 (2H, s, H-3′ and H-5′), 4.79 (1H, td, *J* = 7.8, 6.3 Hz, H-3), 2.79 (2H, d, *J* = 7.8 Hz, H-2), 1.61 (3H, d, *J* = 6.3 Hz, H-4). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 195.9 (C, C-1), 163.5 (C, C-4′), 163.4 (C, C-2′ and C-6′), 105.2 (C, C-1′), 97.1 (CH, C-3′ and C-5′), 76.4 (CH, C-3), 42.6 (CH_2, C-2), 25.3 (CH_3, C-4).

*Silylation Procedure of **9**.* Compound **9** (20 mg, 0.084 mmol) was dissolved in 10 mL of dry Et_2O. After the addition of a large excess of chlorotrimethylsilane (200 μL, 1.6 mmol), followed by 120 μL of dry triethylamine and one drop of DMSO, the reaction mixture was stirred for 2 h at room temperature under N_2. Then, water (10 mL) was added, and the resulting mixture was extracted thrice with Et_2O. The organic layers were combined, dried with anhydrous $MgSO_4$, and concentrated in vacuo to yield a crude mixture containing a tetrasilylated derivative of **9** (84%) which was subjected directly to GC−MS.

*Silylated **9**.* EIMS *m*/*z*: $[C_{22}H_{44}O_5Si_4]^+$ 500 (2), $[M\text{-}Me]^+$ 485 (14), 395 (32), 370 (27), $[M\text{-}C_6H_{15}OSi]^+$ 369 (59), $[M\text{-}2Me_3Si\text{−}H]^+$ 353 (17), 344 (20), 343 (48), $[M\text{-}C_7H_{14}O_2Si]^+$ 342 (100), $[C_5H_{13}OSi]^+$ 117 (42), 75 (25), $[Me_3Si]^+$ 73 (100). RI (DB-5MS) 2111.

In Vitro Cell Assays. *Determination of Splenocyte and Macrophage Viability.* See refs 46 and 47 and the Supporting Information.

Cell Lysosomal Function. See ref 47 and the Supporting Information.

Macrophage Myeloperoxidase Activity Determination. See ref 48 and the Supporting Information.

Statistical Analysis. See the Supporting Information.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00437.

^{1}H and ^{13}C NMR spectra of compounds **1**−**9** and Mosher esters of **1**, (*R*)-**1a**, and (*S*)-**1a**; simulated ^{1}H NMR spectra of compounds **1**, **3**, **7**, and **8**; MS spectra of compounds **1**−**8** and silylated **9**; observed HMBC and NOESY interaction of compounds **1**−**9**; NMR data obtained through full spectral simulations; selected ^{1}H−^{1}H and ^{1}H−^{13}C coupling constants of **1**, **3**, and **8**; and details of in vitro cell assays (PDF)

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Notes

The authors declare no competing financial interest.

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