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ENANTIOMERIC RELATIONSHIPS AND ANTHELMINTIC ACTIVITY OF DYSININ DERIVATIVES FROM *DYSIDEA* MARINE SPONGES

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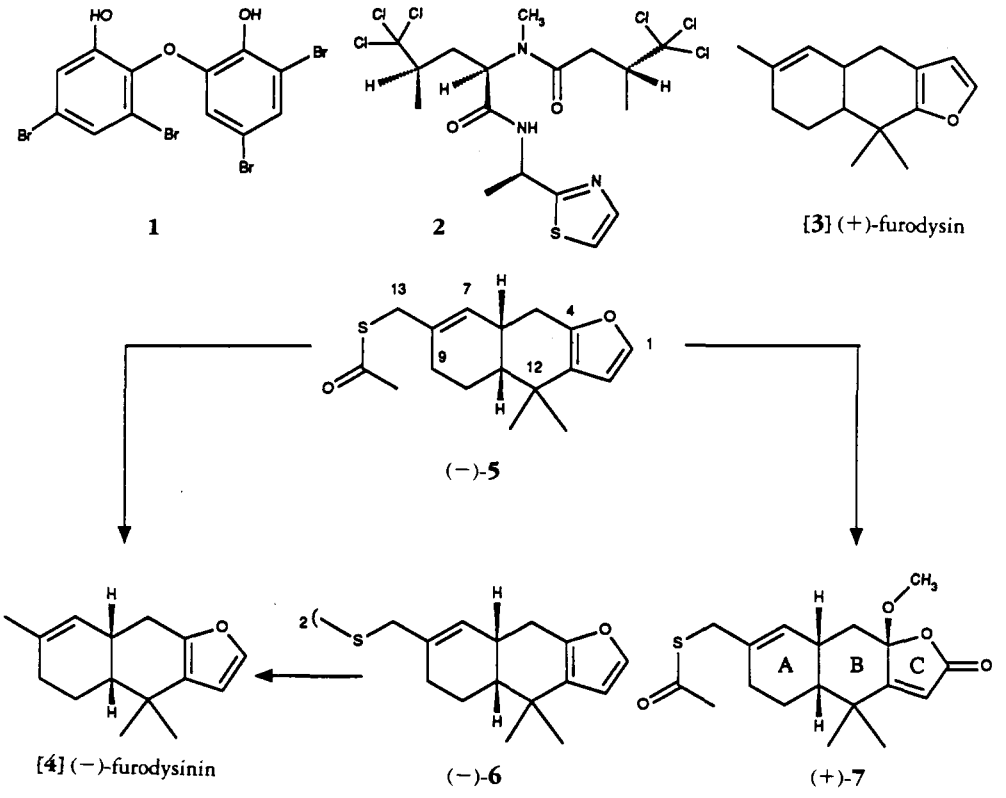
ABSTRACT.—Dysinin-type sesquiterpenes have been isolated during an investigation of the anthelmintic constituents of *Dysidea herbacea* collected from Fiji. These are (−)-(6*R*,11*R*)-furodysinin [**4**], (−)-(6*R*,11*R*)-thiofurodysinin acetate [**5**], (−)-(6*R*,11*R*)-furodysinin disulfide [**6**], and (+)-(4*S*,6*R*,11*R*)-methoxythiofurodysinin acetate lactone [**7**]. Experimental and molecular-mechanics-calculated ^{1}H-nmr *J* values provided insights about the conformation and relative stereochemistry of **7**. Three of these compounds have been previously isolated as (+)-**4** and (+)-**5** with no absolute stereochemistry assigned and **6** (no optical properties reported). Absolute stereochemistry was assigned after establishing a chemical relationship between (−)-**5**, (−)-**6**, and (+)-**7** with (−)-(6*R*,11*R*)-furodysinin [**4**].

Compounds within three vastly different biosynthetic families have been described from *Dysidea herbaceae* Keller (family Dysideidae, order Dictyoceratida). These include polybrominated diphenyl ethers headed by **1**, polychlorinated tetrapeptides such as (−)-dysidenin **2**, or tricyclic furanosesquiterpenes which can be divided into dysin (e.g., (+)-furodysin [**3**]) and dysinin (e.g., (−)-furodysinin [**4**]) types (1,2). An unusual stereochemical situation is presented by the optical properties of the dysinin sesquiterpenes. Kazlauskas *et al.* (3) first reported (+)-**4** and (+)-**5** (without absolute stereochemistry). Guella *et al.* (4) subsequently characterized (−)-**4**, and very recently Richou *et al.* (5), using total synthesis, elucidated the stereochemistry of (−)-**4** as 6*R*,11*R*. We have now isolated (−)-**4**, (−)-**5**, (−)-**6** [recently reported (6) without optical rotation data], and a new metabolite (+)-**7**. This report will consider the absolute stereochemical assignments of these and other dysinin sesquiterpenes along with an analysis of the separate occurrence of members of the two enantiomeric series. Also disclosed will be the results of anthelmintic evaluation of dysinins (−)-**4**, (−)-**5**, (−)-**6**, and (+)-**7**.

RESULTS AND DISCUSSION

This study began several years ago when we observed that extract of *D. herbacea*, collected from Fiji, were very potent in antiparasite tests using *Nippostrongylus brasiliensis*. Bioassay-guided isolations commenced and utilized four collections from Fiji (obtained in the summers of 1985–1987 and 1989) and one from Thailand (obtained in 1988).

The Thailand collection of *D. herbacea* was thin tubular in shape and tan in color. The major component **1** was inactive in anthelminth screens and was accompanied by a mixture of other polybromophenol ethers. By contrast, the *D. herbacea* colonies from Fiji were light purple and tan, massive in size, flat or cup-shaped in appearance. The color of the Fiji collections varies from light purple to tan, which is in contrast to the dark green or ochre previously described for Australian specimens. These color variations may be due to the symbionts of the sponge (7). The extract of the 1989 collection deposited crystalline (−)-furodysinin [**4**], $[\alpha]_D$ −60.9° ($CDCl_3$, $c = 0.01$), mp 55°, during workup. Each of the other Fiji collections yielded crude extracts with ^{13}C resonances characteristic of a -CH_2-S-C(=O)CH_3 group [δ 196 (s), 36 (t), 30 (q)]. Subsequent hplc purification yielded (−)-**5**, $[\alpha]_D$ −34.6°, as a major component, along with a second analogous sesquiterpene, (−)-**6**, $[\alpha]_D$ −27.8°, which possessed a disulfide linkage. This substructure was recognized by comparing the δ 46.3 (t, C-13) of **6** to the best-fit-calculated value of δ 48 for an -SSR substituent. Calculated δ's were derived by adding ^{13}C-nmr shift increments for a -CH_2X array [X ranging from SH



(+11 ppm), SEt (+18 ppm), SSEt (+25 ppm) (8) to a base value of δ 23 (C-13 of **4**). A complete structure was then assembled, and its spectral data turned out to be in agreement with those appearing in the literature for **6** (6) at the conclusion of our study.

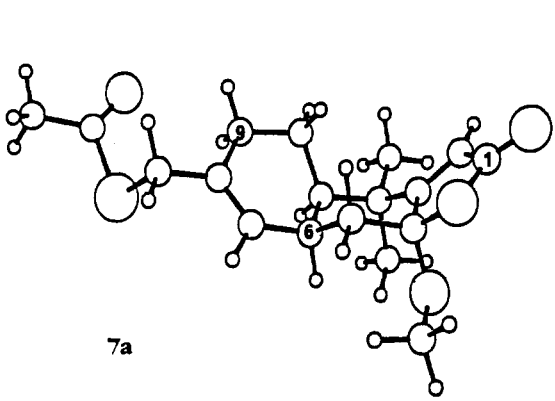
A third more polar compound (+)-**7** ([α]D +135.8°) displayed the ^{13}C-nmr resonances [δ 195 (s), 35 (t), 30 (q)] characteristic of a thioacetate group. Additional resonances at δ 172.8 (s), 169.5 (s), 117.5 (d), 107.4 (s), and 50.5 (q) substantiated a substituted gamma-methoxybutenolide array. The molecular formula of $C_{18}H_{24}O_4S$, from lreims data, the ^{13}C APT spectrum, and additional nmr data indicated the gross methoxythiofurodysinin acetate lactone structure as shown. Chemical verification involved subjecting (−)-**5** to photochemical singlet oxygen reaction, which yielded a single isomer, (+)-**7**.

The relative stereochemistry in **7** of 6*R**,11*R** was established above, but the geometry between the OMe (at C-4) and H-6 was not defined at this point. This relationship was determined to be cis by comparing measured ^3J values ($J_{5,6}=3.9$, $J_{5',6}=13.5$) to J's calculated by molecular mechanics calculations for the two different stereochemical possibilities. Four conformers, summarized in Table 1 (see also **7a–7d**) and consisting of two per family, were generated by varying the C-4 stereochemistry. The conformer **7a**, with cis OMe/H-6, the A ring in a half-chair, and the B ring in a chair conformation, provided a close match between the calculated and experimental J's. Its cis AB ring junction appeared to be locked because the ring-reversed form **7b**, with the B ring in a boat conformation, was predicted to be 5 kcal/mol (21 kJ/mol) higher in energy. Correspondingly higher energies and J's in less agreement with the measured values were calculated for trans OMe/H-6 conformers **7c** and **7d**. These latter two differed by the B ring being flipped between a chair and boat conformation. In summary, **7a** must predominate in the solution phase based on the calculated energies

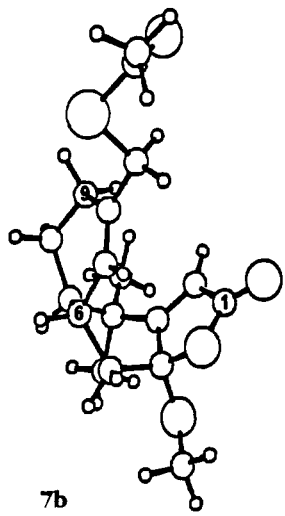
TABLE 1. Energies and Coupling Constants from MACROMODEL or ^{1}H nmr.

Conformer	OMe/H-6 Geometry	Relative energy (kcal/mol)	*J* calcd (Hz)	*J* observed (Hz)
7a	cis	0.0	3.0/12.3	3.9/13.5
7b	cis	4.9	3.4/3.2	
7c	trans	2.3	2.5/4.0	
7d	trans	5.2	6.3/10.5	

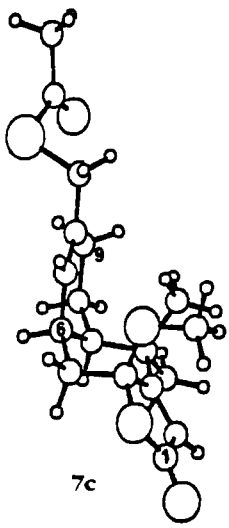
and the agreement between calculated and measured coupling values, and the complete relative stereochemistry of **7** could now be specified as 4*S**,6*R**,11*R**. Analogous to **7** are the known *O*-substituted furodysinin lactones including derivatives (−)-**8**, (+)-**9**, **10**, and (−)-**11** with respectively methoxy (9), ethoxy, and hydroxy (10,11) substituents. Each of the lactones **7**–**10** has a cis geometry between the exocyclic oxygen and H-6. Furthermore, the variation of substituents at C-4 and the consistent observation of only cis alkoxybutenolides either by isolation (9–11) or as synthetic oxidation



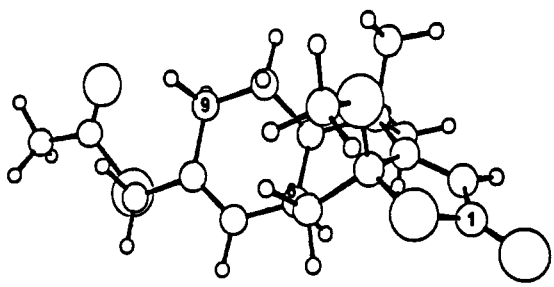
7a



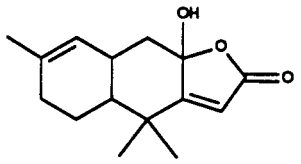
7b



7c



7d



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products of the furan ring (9) are consistent with the trend in calculated cis/trans energies in Table 1. It seems likely that alkoxybutenolide **7** is an extraction artifact arising by an equilibrium-controlled solvent capture of one or more unstable, oxidized dysinin derivatives contained within the sponge. A similar circumstance has been previously discussed by Cartè *et al.* (9) as it was implied that unstable oxidized furanosesquiterpenes were sequestered by nudibranchs associated with *Dysidea* sponges.

Both enantiomeric pairs of dysinin derivatives **4** and **5** have now been isolated. Elucidation by Richou *et al.* (5) of the absolute stereochemistry of (−)-furodysinin [**4**] as 6*R*,11*R* provides a cornerstone for the assignment of absolute stereochemistry of other dysinin derivatives; this is summarized in Table 2. The similar absolute magnitude of rotations recorded in Table 2 for two of the furodysinin samples suggests that the *Dysidea* from Australia and Fiji elaborate antipodes of high purity. We can now assign (−)-**5** as 6*R*,11*R* and (−)-**6** as 6*R*,11*R* because each was separately converted to (−)-**4** using Raney nickel. Likewise the conversion of (−)-**5** to (+)-**7** revealed its absolute stereochemistry as 4*R*,6*R*,11*R*. The dysinin derivatives seem to occur separately in one or the other configurational series according to the organism source. The (+)-**4** has been reported from Australian *Dysidea* (3) whereas (−)-**4** has been isolated from *Dysidea tupha* collected in the East Pyrenean Mediterranean (4) and from our most recent Fiji collection. There are other related dysinin derivatives in the literature but without rotation data. These include (a) furodysinin and furodysin from *Cadlina luteomarginata* collected from British Columbia (12) and (b) furodysinin from *Dysidea etheria* and *Hypselodoris zebra* collected from the Caribbean (13). We also prepared impure (−)-**4** from reduction of precursors obtained from Fijian *Dysidea*. Added to this is the report of (+)-**5** from Australian *Dysidea* (3) and our isolation of (−)-**5** from Fijian *Dysidea*. These enantiospecific relationships also appear to hold if we assume (see Table 2) (a) the thiol (+)-**12** reported from an Australian *Dysidea* (14) is in the enantiomeric series of the disulfide (−)-**6** we isolated from Fijian *Dysidea*, (b) the butenolide (+)-**7** we isolated is in the same stereochemical series as (+)-**9** from *D. tupha* of the East Pyrenean Mediterranean (4), and (c) both (+)-**7** and (+)-**9** (4) are in the series enantiomeric to the butenolides (−)-**8** and (−)-**11** and the hydroperoxide (−)-**13** from Palauan *Chromodorid* nudibranchs (9) or sponges (11). Such a chirality divergence in terpene biosynthesis can be added to another similar situation involving the separate reports of an enantiomeric pair of isothiocyanate sesquiterpenes, epipolasin-A, from sponges (15,16).

Anthelmintic (in vitro) screening conducted with pure compounds (at 50 μg/ml) against *N. brasiliensis* revealed activity for (−)-**4**, (−)-**5**, and (−)-**6** while (+)-**7** was inactive. Follow up in vivo screening against a mixed helminth infection of *Nippostrongylus dubias* and *Hypselodoris nana* revealed no activity at 1000 ppm with (−)-**4** and activity at 444 ppm for a mixture of (−)-**5** and (−)-**6**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a GN-300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Multiplicities of ^{13}C-nmr peaks were determined from APT data. Ms data were obtained on a Finnigan 4000 (6000 LS7 computer system). Hplc was done on a Waters Liquid Chromatograph using 10μ Si gel columns. Rotations were measured on a Perkin-Elmer 141 polarimeter.

TABLE 2. Stereochemical Variations and Sources of Dysinin Derivatives.

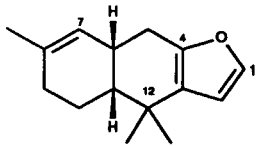
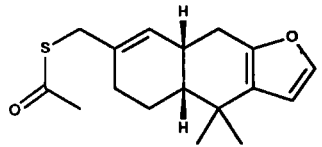
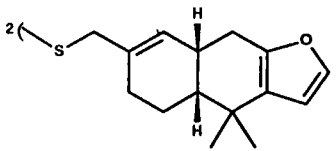
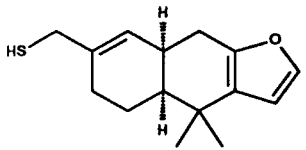
Compound	Configuration of				Source	Reference
	[α]D	C-4	C-6	C-11		
 [4][a] furodysinin	−48[b]	—	*R*	*R*	*Dysidea tupha* (Mediterranean)	Guella *et al.* (4)
	+64[b]	—	*S*	*S*	*Dysidea herbacea* (Australia)	Kazlauskas *et al.* (3)
	−25[b]	—	*R*	*R*	Synthetic	Richou *et al.* (5)[a]
	−61[b]	—	*R*	*R*	*D. herbacea* (Fiji)	this work
	−10	—	*R*	*R*	Synthetic	this work
 **5**	−35	—	*R*	*R*	*D. herbacea* (Fiji)	this work
	+52	—	*S*	*S*	*D. herbacea* (Australia)	Kazlauskas *et al.* (3)
 **6**	−28	—	*R*	*R*	*D. herbacea* (Fiji)	this work
 **12**	+49	—	*S*	*S*	*Dysidea avara* (Australia)	Capon and MacLeod (14)

TABLE 2. Continued.

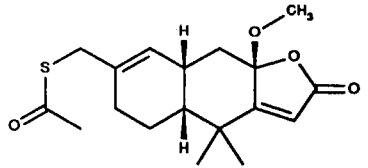
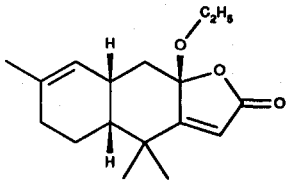
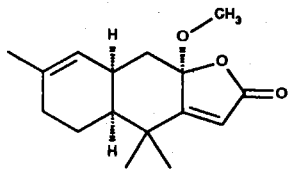
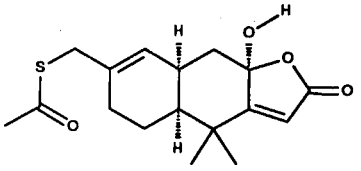
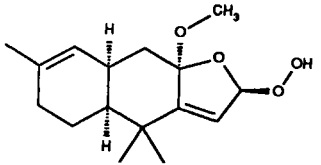
 **7**	+136	*S*	*R*	*R*	*D. herbacea* (Fiji)	this work
 **9**	+222	*S*	*R*	*R*	*D. tupha* (Mediterranean)	Guella *et al.* (4)
 **8**	−85	*R*	*S*	*S*	*Chromodoris funera* (Palau)	Cartè *et al.* (9)
 **11**	−178	*R*	*S*	*S*	*Dysidea* sp. (Palau)	Cartè *et al.* (11)

TABLE 2. Continued.

 13	−64	*R*	*S*	*S*	*C. funera* (Palau)	Cartè *et al.* (9)

[a]Absolute stereochemistry of (−)-**4** assigned by Richou *et al.* (5) (total synthesis); other assignments are inferred by synthetic interconversion to (−)-**4** or by arguments in the text.

[b]Solvent = $CDCl_3$ or $CHCl_3$.

COMPUTATIONAL METHODS.—Computer modeling was carried out with the MACROMODEL program (version 1.5) on a Vax 11/750 computer with an Evans and Sutherland (PS 300) picture system. Molecular mechanics calculations were performed with the MM2 force field with a distance-dependent dielectric. Structures were energy-minimized with the Block Diagonal Newton Raphson algorithm in Cartesian coordinate space until the rms energy gradient was less than 0.04 kJ/mol Å. Vicinal coupling constants in the candidate conformations were calculated in MACROMODEL with the Altona coupling equation (17).

2D NMR PROCEDURES.—Standard pulse sequences were used for the homo COSY and the hetero COSY experiments (18).

ISOLATION PROCEDURES.—The fresh *D. herbacea* (6.8 kg) was cut into small pieces and soaked in CH_2Cl_2 for 24 h followed by MeOH for 24 h. Voucher specimens and their underwater photos are available from the senior author. The solvents were decanted, and the oils were concentrated (yield of MeOH extract = 14.27 g). The oils were combined and a portion of the oil was successively partitioned between equal volumes (500 ml of aqueous MeOH, % adjusted to produce a biphase solution) and a solvent series of hexanes (4.38 g), CCl_4 (1.31 g), and CH_2Cl_2 (1.36 g). The hexanes partition fraction was then chromatographed (normal phase flash cc) using EtOAc-hexanes (5:95) with successive increases in EtOAc until pure EtOAc was attained. The fractions containing compounds of similar polarity were monitored by tlc and ^{13}C nmr. The fractions which displayed low-field signals in the ^{13}C-nmr spectra were combined and further purified via preparative normal phase hplc [Regis 10μ-silica column, hexanes-EtOAc (95:5)] to yield (% based on solvent partition fraction) (−)-thiofurodysinin acetate [**5**] (3.07%), $[\alpha]_D$ −34.6° (c = 0.028, C_6H_6), and (−)-furodysinin disulfide [**6**] (1.66%), $[\alpha]_D$ −27.8° (c = 0.009, C_6H_6). The CCl_4 partition fraction was likewise chromatographed (normal phase flash cc) using CCl_4-EtOAc (90:10) with successive increases in EtOAc until pure EtOAc was obtained. The fractions were monitored by tlc and ^{13}C nmr. The fractions which displayed low-field signals in the ^{13}C-nmr spectra were combined and further purified via preparative reversed-phase hplc [10μ ODS column, MeOH-H_2O (90:10)] to yield (+)-methoxythiofurodysinin acetate lactone [**7**] (1.73%), $[\alpha]_D$ +135.8° (c = 0.015, C_6H_6).

OXIDATION OF (−)-THIOFURODYSININ ACETATE [**5**] WITH SINGLET OXYGEN.—A solution of (−)-thiofurodysinin acetate [**5**] (45.5 mg) in CH_2Cl_2-MeOH (3:1) containing 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine under an atmosphere of O_2 was irradiated with a 275-W incandescent lamp for 1.5 h. The solvent was then evaporated, and the residue was taken up in hexanes-EtOAc (5:1) and passed through a short column of Si gel. The sample was further purified by preparative reversed hplc [10μ ODS column, MeOH-H_2O (90:10)], yielding (+)-methoxythiofurodysinin acetate lactone [**7**] (17.3 mg).

REDUCTION OF (−)-THIOFURODYSININ ACETATE [**5**].—Raney nickel suspension (1.0 g) was added to an EtOH solution of (−)-thiofurodysinin acetate [**5**] (0.2143 g). The mixture was stirred for 10 min and filtered through Celite. The resulting product was then purified by normal phase hplc [Regis 10μ-silica column, hexanes-EtOAc (85:15)] to yield (−)-furodysinin [**4**] (0.1348 g).

REDUCTION OF (−)-FURODYSININ DISULFIDE [**6**].—Raney nickel suspension (40.0 mg) was added to an EtOH solution of (−)-thiofurodysinin disulfide [**6**] (10.0 mg). The mixture was stirred for 10 min and filtered through Celite. The resulting product was then purified by normal phase hplc [Regis 10μ-silica column, hexanes-EtOAc (85:15)] to yield (−)-furodysinin [**4**] (8.87 mg).

(+)-METHOXYTHIOFURODYSININ ACETATE LACTONE [**7**].—Viscous oil $[\alpha]_D$ +135° (c = 0.015, C_6H_6); nmr (shifts in ppm from TMS, assignments based on assessing the number of attached protons and COSY data) (^{13}C δ's at 75 MHz, ^1H δ's and J values at 300 MHz) [C_6H_6-$CDCl_3$ (3:1)] C-1 172, C-2 117, H-2 5.55 (s, 1H), C-3 169.5, C-4 107.4, C-5 39.6, H-5 2.34 (dd, J = 13.8, 3.9), 1.23 (t, J = 13.5), C-6 30.5, H-6 2.55 (m), C-7 127.5, H-7 5.41 (d, J = 4.8), C-8 133.8, C-9 28.2, H-9 1.77 (dd, J = 5.4, 3.3), C-10 18.4, H-10 1.36 (m), 0.78 (m), C-11 47.5, H-11 1.32 (m), C-12 38.7, C-13 35.4, H-13 3.23 (s, 2H), C-14 195.3, C-15 30.4, H-15 2.03 (s, 3H), C-16, -17 25.3, 25.8, H-16, -17 1.05 (s, 3H), 0.88 (s, 3H), C-18 50.5, H-18 2.03 (s, 3H). Selected nmr COSY data: ^1H-^1H [C_6H_6-$CDCl_3$ (3:1)] regular H-5,-5′ ⟷ H-6, H-6 ⟷ H-11, H-6 ⟷ H-7, H-9,-9′ ⟷ H-10,-10′, H-10,-10′ ⟷ H-11; long range H-7 ⟷ H-9,-9′; lreims *m/z* (%) 336 (13), 261 (68), 167 (100).

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