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Tripartilactam, a Cyclobutane-Bearing Tricyclic Lactam from a *Streptomyces* sp. in a Dung Beetle's Brood Ball

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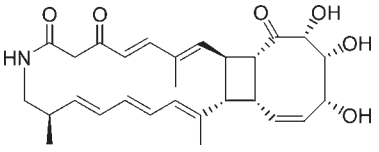
ABSTRACT



Tripartilactam, a structurally unprecedented cyclobutane-bearing tricyclic lactam metabolite, was discovered from *Streptomyces* sp. isolated from a brood ball of the dung beetle, *Copris tripartitus*. The structure of this compound was elucidated by the combination of NMR, MS, UV, and IR spectroscopy and multistep chemical derivatization. Tripartilactam was evaluated as a Na^+/K^+ ATPase inhibitor (IC_{50} = 16.6 μg/mL).

Diverse insect ecosystems have recently drawn significant attention as a new source of natural products with pharmaceutical potential.[1] This attention is partly due to insect–microbial mutualisms being mediated by microbial secondary metabolites with selective activity, as shown in the ecologically well-defined symbiotic systems of the southern pine beetle (*Dendroctonus frontalis*)[2] and the fungus-growing ant (*Apterostigma dentigerum*).[3] However, the potential of microorganisms discovered in insect ecosystems is now being explored because most such microbial communities are unexplored niches. The recent study of the mud dauber, *Sceliphron caementarium*, led to the discovery of a novel antifungal polyene macrocyclic lactam, sceiliphrolactam, although its ecological role has not been completely determined.[4] Biomedical investigation of the gut microbiota of a mantis isolated a fungal species, *Daldinia eschscholzii*, that produces new immunosuppressive polyketides known as dalesconols.[5] Even a fungal strain isolated from the nest of the fungus-growing ant (*A. dentigerum*) yielded a new polyketide glycoside.[6]

The dung beetle, *Copris tripartitus*, is a soil-dwelling insect, the life cycle of which is tightly dependent on the feces of herbivores.[7] The brood balls mainly composed of feces are expected to harbor extensive microbial communities. Our recent selective isolation of actinomycetes from

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(1) (a) Crawford, J. M.; Clardy, J. *Chem. Commun.* **2011**, *47*, 7559. (b) Bode, H. B. *Angew. Chem., Int. Ed.* **2009**, *48*, 6394. (c) Kaltenpoth, M. *Trends Microbiol.* **2009**, *17*, 529.

(2) (a) Scott, J. J.; Oh, D.-C.; Yuceer, M. C.; Klepzig, K. D.; Clardy, J.; Currie, C. R. *Science* **2008**, *322*, 63. (b) Oh, D.-C.; Scott, J. J.; Currie, C. R.; Clardy, J. *Org. Lett.* **2009**, *11*, 633.

(3) Oh, D.-C.; Poulsen, M.; Currie, C. R.; Clardy, J. *Nat. Chem. Biol.* **2009**, *5*, 391.

(4) (a) Oh, D.-C.; Poulsen, M.; Currie, C. R.; Clardy, J. *Org. Lett.* **2011**, *13*, 752. (b) Poulsen, M.; Oh, D.-C.; Clardy, J.; Currie, C. R. *PLoS One* **2011**, *6*, e16763.

(5) Zhang, Y. L.; Ge, H. M.; Zhao, W.; Dong, H.; Xu, Q.; Li, S. H.; Li, J.; Zhang, J.; Song, Y. C.; Tan, R. X. *Angew. Chem., Int. Ed.* **2008**, *47*, 5823.

(6) Freinkman, E.; Oh, D.-C.; Scott, J. J.; Currie, C. R.; Clardy, J. *Tetrahedron Lett.* **2009**, *50*, 6834.

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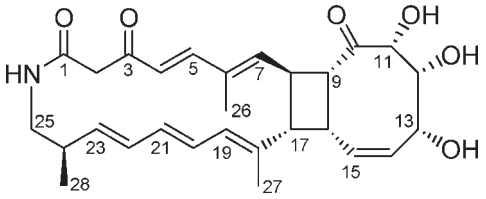
the beetle's brood balls revealed the diversity of the actinomycetous population and their potential as a new bioactive chemotype.[8]

We have continuously investigated the chemical and biological diversity of the actinomycetes isolated from dung brood balls in the search for structurally novel bioactive compounds. In our chemical analysis of the actinomycetes' secondary metabolites, we discovered a *Streptomyces* strain, SNA112,[9] that produces a compound with a distinct UV spectrum (λ_{max} at 277 nm). Further characterization of this compound's structure led us to identify an unprecedented cyclobutane-bearing tricyclic lactam, tripartilactam (**1**). Herein, we report the isolation, structural determination, stereochemistry, and biological activity of this novel tricyclic lactam.



1

Tripartilactam (**1**)[10] was obtained as a yellowish powder. The molecular formula, $C_{28}H_{35}NO_6$, was deduced based on ^{1}H and ^{13}C NMR spectral data (Table 1) and FAB high-resolution mass spectrometry (observed $[M+H]^+$ at m/z: 482.2542, calculated: 482.2543). The ^{13}C NMR spectrum displayed a polyunsaturated signature with 12 carbon signals from δ_C 147.0 to 121.7. Three carbonyl carbons were observed at δ_C 210.0, 191.2, and 164.4. Based on the carbon chemical shift and the IR absorption at 1677 cm^{-1}, the carbonyl ^{13}C peak at δ_C 164.4 was assigned as an amide carbon. In the oxygenated carbon region, three carbons were identified at δ_C 78.7, 74.2, and 70.2. The upfield region of the ^{13}C NMR spectrum displayed signals for 10 aliphatic carbons (Table 1).

The ^{1}H NMR spectrum contained signals for a polyunsaturated feature consistent with 10 olefinic protons from 7.10 to 5.23 ppm and two allylic methyl groups (δ_H 1.49 and 1.26), accounting for six double bonds indicated by the 12 olefinic carbons in the ^{13}C NMR spectrum. The amide functionality was also confirmed by the observation of the NH signal at δ_H 7.99. In addition, tripartilactam showed three carbinol protons (δ_H 4.52, 4.02, 3.82) along with three D_2O exchangeable proton signals (δ_H 5.29, 4.93, 4.63). The presence of hydroxy moieties was also supported by the broad IR absorption at 3361 cm^{-1}. In the aliphatic region, one doublet representing a methyl group (δ_H 0.93) and nine protons between δ_H 3.54 and 2.32 were found.

Table 1. NMR Data for Tripartilactam (**1**) in DMSO-d_6

C/H	δ_H[a]	mult (J in Hz)	δ_C[b]	
1			164.4	C
2a	3.47	d (11.5)	51.4	CH_2
2b	2.96	d (11.5)		
3			191.2	C
4	6.03	d (15.5)	121.7	CH
5	7.10	d (15.5)	147.0	CH
6			135.4	C
7	5.70[c]	m	145.6	CH
8	2.57	ddd (12.0, 10.0, 10.0)	38.0	CH
9	2.38	m	48.6	CH
10			210.0	C
11	3.82	br s	70.2	CH
11-OH	5.29	br d (2.5)		
12	4.02	m	78.7	CH
12-OH	4.93	br d (4.0)		
13	4.52	dd (7.5, 3.5)	74.2	CH
13-OH	4.63	br d (7.5)		
14	5.71[c]	m	130.0	CH
15	5.76	ddd (10.0, 5.0, 2.5)	125.0	CH
16	3.54	m	44.4	CH
17	2.43	ddd (10.0, 4.0, 2.5)	52.2	CH
18			134.3	C
19	5.39	d (11.0)	130.1	CH
20	6.12	dd (14.5, 11.0)	125.7	CH
21	5.68	dd (14.5, 10.0)	132.5	CH
22	6.02	dd (15.0, 10.0)	130.3	CH
23	5.23	dd (15.0, 9.0)	137.8	CH
24	2.32	m	38.2	CH
25a	3.25	m	44.1	CH_2
25b	2.90	ddd (13.0, 4.0, 4.0)		
25-NH	7.99	dd (8.0, 4.0)		
26	1.26	s	13.1	CH_3
27	1.49	s	12.9	CH_3
28	0.93	d (6.5)	19.0	CH_3

[a] 500 MHz. [b] 125 MHz. [c] Overlapped.

The overall analysis of the ^{13}C and ^{1}H NMR spectra and the IR spectrum revealed that this compound bears six double bonds and three carbonyl groups, which account for 9 out of 12 unsaturation equivalents calculated from the molecular formula. Thus, tripartilactam (**1**) must be a tricyclic compound.

Interpretation of the gHSQC and gCOSY NMR spectra established the isolated spin system from C-19 to 25-NH, including the connectivity between C-28 (aliphatic methyl group; δ_C 19.0–δ_H 0.93) and C-24 (methine; δ_C 38.2–δ_H 2.32) (Figure 1). The COSY correlations among the aliphatic methines (C-8 δ_C 38.0–δ_H 2.57; C-9 δ_C 48.6–δ_H 2.38; C-16 δ_C 44.4–δ_H 3.54; C-17 δ_C 52.2–δ_H 2.43) surprisingly revealed the existence of a cyclobutane ring. Further extension of this spin system includes C-7, C-15, and C-14 according to the COSY and the HMBC correlations. The triol moiety (C-11 to C-13), a discrete double bond (C-4 and C-5), and an isolated methylene (C-2) were

(7) Fincher, G. T. *J. Ga. Entomol. Soc.* **1981**, *16*, 316.

(8) Kim, S.-H.; Ko, H.; Bang, H.-S.; Park, S.-H.; Kim, D.-G.; Kwon, H. C.; Kim, S. Y.; Shin, J.; Oh, D.-C. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5715.

(9) The bacterial strain SNA112 was isolated from a dung brood ball of *C. tripartitus* on Czapek-Dox agar supplemented with cycloheximide. The 16S rDNA analysis resulted in placing SNA112 in the genus *Streptomyces* because it is most closely related to *Streptomyces corchorusii* (99% identity).

(10) Tripartilactam (**1**): yellow powder; $[\alpha]_D = -128$ (c 0.20, MeOH); IR (neat) ν_{max} 3361, 2922, 1723, 1677, 1581 cm^{-1}; UV (MeOH) λ_{max} (log ε) 277 (4.51) nm; NMR spectral data, see Table 1; HR-FAB MS $[M+H]^+$ m/z 482.2542 ($C_{28}H_{35}NO_6$) calcd $[M+H]^+$ 482.2543.

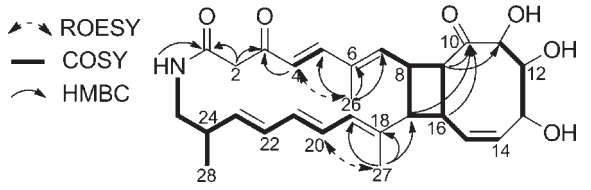


Figure 1. Key ROESY, COSY, and HMBC correlations establishing the planar structure of tripartilactam (**1**).

also identified, based on the $^1H-^1H$ couplings in the COSY spectrum (Figure 1).

The long-range heteronuclear correlations in the gHMBC spectrum verified the connectivities of these spin systems. The coupling from H_2-2 to the carbonyl carbons C-1 and C-3 located the methylene C-2 between these carbonyl groups. Similarly, the HMBC correlations from H-4 to C-3 and from H_3-26 to C-5, C-6, and C-7 established the connectivity from C-1 to the cyclobutane ring. The triol partial structure was found to be connected to the cyclobutane ring with the ketone carbon as a linker based on the HMBC correlations between H-9 and both C-10 and C-11. This assigned location is also further supported by the long-range correlations from H-16 and H-17 to C-10. The allylic methyl signal (H_3-27) generated strong HMBC correlations to C-17, C-18, and C-19, connecting the chain structure from C-20 to 25-NH to the cyclobutane ring. The presence of an 18-membered macrocyclic ring was confirmed by the HMBC correlation between 25-NH and C-1. Although no correlations in the COSY, HMBC, and ROESY spectra in three different solvents (DMSO-d_6, pyridine-d_5, and CD_3OD) were observed to directly connect C-13 to C-14, this linkage was deduced based on the molecular formula; this linkage completed the planar structure of **1** bearing a cyclooctene ring.

The double bond geometries in **1** were assigned based on the analysis of $^1H-^1H$ coupling constants and ROESY correlations (Figure 1). The large coupling constant between H-4 and H-5 (15.5 Hz) established the 4*E* configuration. The 6*E* configuration was required by the ROESY correlations between H_3-26 and H-4. The $^1H-^1H$ *cis*-coupling (10.0 Hz) between H-14 and H-15 determined 14*Z*. The H-20-H-21 *trans* coupling constant (14.5 Hz) established 20*E*. The 22*E* configuration was assigned based on the large coupling between H-22 and H-23 (15.0 Hz). Finally, the 18*E* configuration was deduced by the ROESY correlation between H_3-27 and H-20.

Because the coupling constants between the protons in cyclobutane are not sufficiently specific to determine the relative configuration in the ring,[11] the relative configuration of the cyclobutane ring was established by careful analysis of the transannular ROESY correlations (Figure 2).

(11) Pretsch, E.; Bühlmann, P.; Affolter, C. *Structure Determination of Organic Compounds-Tables of Spectral Data*; Springer: New York, 2000; p 176.

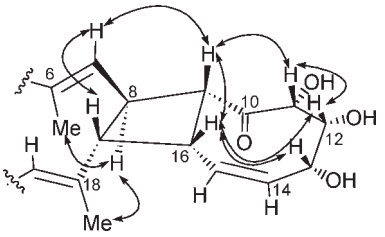


Figure 2. Key ROESY correlations of the cyclobutane and cyclooctene ring in tripartilactam (**1**).

The ROESY correlations in the cyclobutane and cyclooctene rings indicated that the relative configurations of C-8, C-9, C-11, C-12, C-13, C-16, and C-17 were 8*R**, 9*R**, 11*R**, 12*R**, 13*R**, 16*R**, and 17*S**, respectively.

The absolute configuration of the C-24 chiral center was determined by multistep chemical degradation (Figure 3).

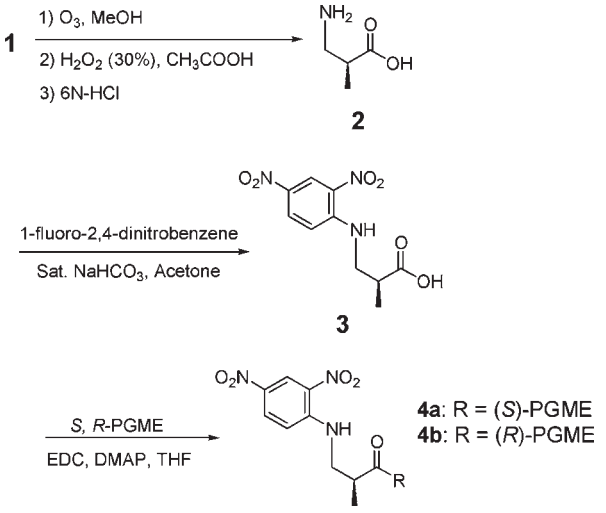


Figure 3. Multistep chemical degradation and derivatization for the determination of the absolute configuration of the C-24 chiral center.

Tripartilactam was subjected to ozonolysis and acid hydrolysis to yield 3-amino-2-methyl-propioinic acid (**2**). This β-amino acid (**2**) was derivatized with the Sanger reagent to generate a product (**3**) with improved UV absorption and retention in reversed-phase HPLC. Compound **3** was further derivatized with (*S*)- and (*R*)-phenylglycine methyl ester (PGME) to furnish (*S*)- and (*R*)-PGME amide products (**4a** and **4b**, respectively). Analysis of the ^1H NMR spectra allowed the assignment of the proton chemical shifts in **4a** and **4b**. Calculation of the $\Delta\delta_{S-R}$ values clearly established the absolute configuration of C-24 as *R* based on the consistent distribution of the signs (Figure 4).[12]

(12) Yabuuchi, T.; Kusumi, T. *J. Org. Chem.* **2000**, *65*, 397.

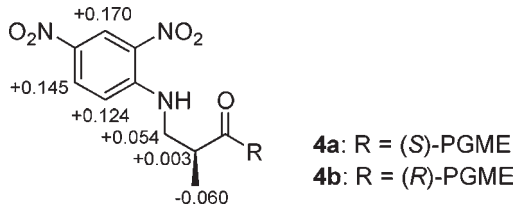


Figure 4. $\Delta\delta_{S\text{-}R}$ values in ppm for the *S*- and *R*-PGME amide products (**4a** and **4b**) in CD_3OD.

The absolute configurations of the stereogenic centers in cyclobutane and cyclooctene were determined by the application of the modified Mosher method using (*R*)- and (*S*)-α-methoxy-(trifluoromethyl) phenyl acetyl chloride (MTPA-Cl). Since tripartilactam bears three consecutive hydroxy groups at C-11, C-12, and C-13, it was challenging to generate mono-MTPA esters. After a significant amount of effort on reaction optimization, we tried a short reaction time (5 min) and succeeded in obtaining mono-(*S*)- and (*R*)-MTPA ester selectively at the alcohol of C-13 (**5a** and **5b**). Analysis of ^{1}H NMR and COSY spectral data for these MTPA esters allowed the assignment of the $\Delta\delta_{S\text{-}R}$ values, which sufficiently established the absolute configuration of C-13 as 13*R* (Figure 5).[13] Based on the relative configuration, the absolute configurations of the chiral centers C-8, C-9, C-11, C-12, C-16, and C-17 were determined as 8*R*, 9*R*, 11*R*, 12*R*, 16*R*, and 17*S*.

The biological activity of **1** was evaluated first in cell-based antimicrobial and anticancer assays, in which no significant inhibition against various pathogenic bacterial and fungal strains was observed (see Supporting Information (SI)). This compound was also inactive against the A549 lung cancer cell line and the HCT116 colon cancer cell line at 100 μg/mL. However, tripartilactam was observed to be a moderate inhibitor of Na^+/K^+ ATPase, with an IC_{50} value of 16.6 μg/mL.

The structure of **1** is unique because of the existence of cyclobutane linking the 8- and 18-membered rings. This ring structure could be formed by a photochemically derived [2 + 2] cycloaddition reaction[14] of the two double bonds at positions 8 and 16 in a putative macrocyclic lactam precursor (see SI Figure S3).

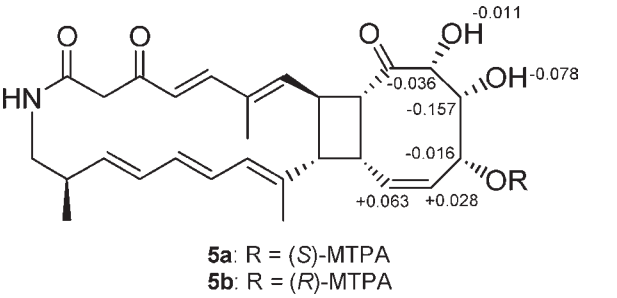


Figure 5. $\Delta\delta_{S\text{-}R}$ values in ppm for the *S*- and *R*-MTPA esters (**5a** and **5b**) in DMSO-d_6.

Four- and eight-membered bicyclic structures have been reported in terpenoids from terrestrial plants[15] and marine soft corals.[16] However, the 4-, 8-, and 18-membered tricyclic structure of **1** is an unprecedented carbon skeleton, to the best of our knowledge.

The discovery of tripartilactam with a novel carbon scaffold provides additional evidence that microbial communities of insect habitats could be promising sources of structurally novel bioactive natural products.

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Supporting Information Available. Experimental section, bioassay data, a proposed biosynthetic pathway of **1**, and NMR spectra of **1**, **3**, **4a**, **4b**, **5a**, and **5b**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(13) Seco, J. M.; Quiñoá, E.; Riguera, R. *Tetrahedron: Asymmetry* **2001**, *12*, 2915.

(14) We propose that this [2 + 2] cycloaddition is photochemically derived because we could not find any precedent example of enzymatic [2 + 2] cycloaddition thus far to the best of our knowledge. However, we could not completely exclude the possibility of the enzymatic involvement in this thermally forbidden reaction. For the general mechanism of photochemical [2 + 2] cycloaddition, see: Carruthers, W. *Cycloaddition Reactions in Organic Synthesis*; Pergamon Press: 1991; p 322. For another possible cyclobutane formation mechanism by unusual oxidative cyclization or radical cascading proposed in the ladderane biosynthesis, see: Rattary, J. E.; Strous, M.; Op den Camp, H. J. M.; Schouten, S.; Jetten, M. S. M.; Damsté, J. S. S. *Biol. Direct* **2009**, *4*, 8.

(15) (a) Jacobsson, U.; Kumar, V.; Saminathan, S. *Phytochemistry* **1995**, *39*, 839. (b) Macleod, J. K.; Rasmussen, H. B. *Phytochemistry* **1999**, *50*, 105.

(16) (a) Sung, P.-J.; Chuang, L.-F.; Kuo, J.; Chen, J.-J.; Fan, T.-Y.; Li, J.-J.; Fang, L.-S. *Chem. Pharm. Bull.* **2007**, *55*, 1296. (b) Groweiss, A.; Kashman, Y. *Tetrahedron* **1983**, *39*, 3385.