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Javaniside, a novel DNA cleavage agent from *Alangium javanicum* having an unusual oxindole skeleton

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Bioassay-guided fractionation of an extract prepared from *Alangium javanicum* using an assay to monitor Cu^{2+}-dependent DNA strand scission led to the isolation of javaniside, a new type of oxindole glycoside.

The recognition that DNA may serve as a target for small molecules in the initiation of cellular disorders and in the therapy of certain diseases has served to arouse increased interest in the interaction of such species with DNA. Non-covalent binding interactions, such as intercalation by planar aromatic species, covalent binding interactions, such as that exemplified by Cisplatin, and DNA strand scission have all been documented.[1] DNA strand scission may occur through processes such as oxidative transformation of the deoxyribose ring, aromatic nucleobase alkylation or oxidation, or phosphodiester backbone hydrolysis.[2]

As a consequence of the clinical utility of DNA cleavage agents such as bleomycin,[3] considerable effort has been made to identify and characterize naturally occurring molecules capable of mediating DNA strand scission, as such species may serve as lead structures for the development of novel anti-tumor drugs. Natural products shown to have such activity to date include resorcinols,[4] flavonoids,[5] stilbenes,[6] quinones,[7] and cyclopeptides.[8] Here, we describe the isolation and structural elucidation of javaniside, a novel oxindole glycoside from *Alangium javanicum* that induces DNA strand scission in the presence of Cu^{2+}.

An organic extract prepared by soaking leaves of *A. javanicum*† in 1:1 CH_2Cl_2–methanol at room temperature overnight was fractionated initially on a polyamide 6S column. The column was washed successively with H_2O, 1:1 MeOH–H_2O, 4:1 MeOH–CH_2Cl_2, 1:1 MeOH–CH_2Cl_2, and then 9:1 MeOH–NH_4OH. The first two fractions exhibited the most potent Cu^{2+}-dependent DNA cleavage activity; these were combined and fractionated on a C_{18} open column using CH_3OH–H_2O mixtures for elution. The 7:3 CH_3OH–H_2O fraction possessed the most potent DNA cleavage activity and was fractionated further by C_{18} reversed-phase HPLC (linear gradient from 1:19 → 2:3 CH_3CN–H_2O over a period of 35 min at 4.0 ml min^{-1}, UV monitoring at 254 nm). One active fraction was obtained; repeated purification by reversed-phase HPLC afforded pure compound **1**. The yield of **1** from the initial crude extract was 0.53% by weight.

Javaniside, **1** (Fig. 1), was obtained as a colorless amorphous powder and gave rise to a signal corresponding to $[M + H]^+$ at *m*/*z* 515.2015 (positive ion HR-FABMS), establishing that the molecular formula is $C_{26}H_{30}N_2O_9$. In the downfield region of the ^1H NMR spectrum of **1**, eight aromatic or olefinic proton resonances were observed: four of these protons belonged to an aromatic ring [δ 7.30 (J = 7.5 Hz), 7.07 (J = 7.5, 1.2 Hz), 7.25 (J = 7.5, 1.2 Hz), and 6.90 (J = 7.5 Hz)], one was a deshielded olefinic proton [δ 7.38 (J = 2.3 Hz)], and the other three were indicative of a terminal vinyl group. Additionally, one acetal proton [δ 5.42 (d, J = 1.8 Hz)] also resonated in this region. Moreover, in addition to seven sugar ring proton peaks in the high field region of the spectrum, another nine proton signals were also observed (Table 1). The ^{13}C NMR and DEPT spectra revealed the existence of two carbonyl groups (δ 180.9 and 165.8), six aromatic carbons (δ 143.6, 130.0, 129.5, 123.9, 123.8, and 110.9), two aliphatic terminal vinyl carbons (δ 133.8 and 120.4), and one trisubstituted double bond (δ 148.2 and 108.9). In addition to six sugar resonances (δ 99.5, 78.3, 77.9, 74.8, 71.5, and 62.6), the spectra also suggested the presence of three methylenes, four methines, and one quaternary carbon resonating in the range 20–100 ppm. Careful analysis of ^1H–^1H DQ-COSY and HMQC spectra led to the assignment of all proton and carbon resonances. Furthermore, 2D DQ-COSY and TOCSY experiments allowed the assignment of four separated spin–spin coupling systems reflected in structure **1**. Subsequently, the multi-bond ^1H–^{13}C correlations derived from HMBC experiments (Fig. 2) provided key connectivity information and led to the establishment of the structure of **1** as an unusual oxindole alkaloid with a monoterpene fragment and glucose moiety (Fig. 1). The sugar was

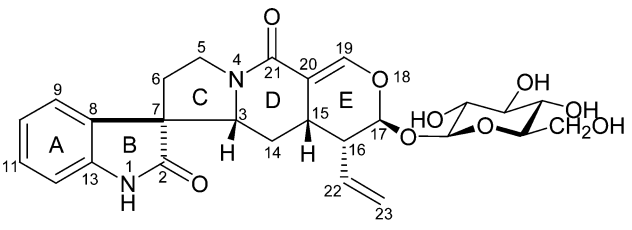

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Fig. 1 Structure of javaniside, illustrating its assigned relative stereochemistry.

Table 1 ^1H and ^{13}C NMR spectral data for javaniside in CD_3OD

C	δ_H[a] (multiplicity, *J*/Hz)	δ_C[b]
2	—	180.9
3	4.09 (dd, 11.6, 3.0)	65.5
5_α	4.04 (ddd, 11.4, 10.5, 7.4)	45.6
5_β	3.76 (br t, 11.3)	—
6_α	2.23 (dd, 13.2, 7.4)	33.4
6_β	2.40 (ddd, 13.2, 11.2, 10.5)	—
7	—	58.0
8	—	129.5
9	7.30 (br d, 7.5)	123.9
10	7.07 (td, 7.5, 1.2)	123.8
11	7.25 (td, 7.5, 1.2)	130.0
12	6.90 (br d, 7.5)	110.9
13	—	143.6
14_α	1.27 (td, 12.2, 12.4)	27.0
14_β	1.37 (dt, 12.2, 3.8)	—
15	2.94 (dddd, 12.4, 5.5, 3.8, 2.4)	28.7
16	2.56 (ddd, 9.8, 5.5, 1.8)	44.6
17	5.42 (d, 1.8)	97.3
19	7.38 (d, 2.3)	148.2
20	—	108.9
21	—	165.8
22	5.49 (dt, 16.8, 10.0)	133.8
23	5.16 (dd, 10.0, 1.8)	120.4
23	5.19 (dd, 16.8, 1.8)	—
1′	4.64 (d, 8.0)	99.5
2′	3.14 (dd, 9.0, 8.0)	74.8
3′	3.36 (m)	77.9
4′	3.26 (m)	71.5
5′	3.28 (m)	78.3
6′	3.63 (dd, 12.0, 5.6)	62.6
6′	3.83 (dd, 12.0, 2.0)	—

[a] Determined at 500 MHz. [b] Determined at 125 MHz.

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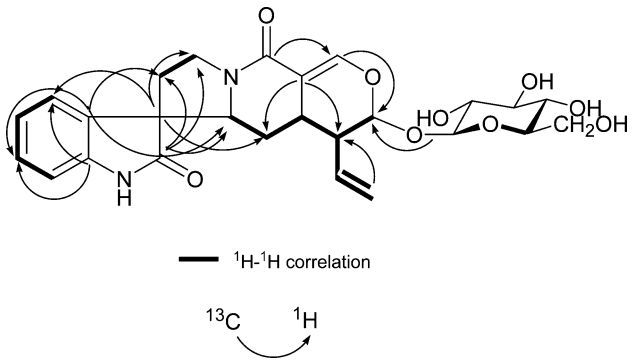

Fig. 2 ^{1}H–^{1}H and ^{1}H–^{13}C long range HMBC correlations for javaniside.

identified unambiguously as glucose by ^{1}H and ^{13}C NMR spectroscopy. It is drawn as the naturally abundant D-isomer, albeit without direct proof.

The stereochemistry of **1** was determined carefully through the analysis of all proton coupling constants and NOE effects. All coupling constants deduced from ^{1}H, ^{1}H–^{1}H COSY, and ^{1}H–^{1}H TOCSY spectra indicated that each ring of **1** adopts the relative configuration illustrated in Fig. 1.[9–13] The coupling constants for H_{16}–H_{17} (J = 1.8 Hz) and H_{15}–H_{16} (J = 5.5 Hz) strongly suggested the β/β/α orientation of H_{15}, H_{16}, and H_{17}. The β configuration of H_3 was supported by the coupling constants between H_3 and H_{14} ($J_{3,14\alpha}$ = 11.6 Hz, $J_{3,14\beta}$ = 3.0 Hz). Moreover, NOE signals from NOESY experiments provided additional clues as to the stereochemistry of **1** (Fig. 3). The NOE correlations between H_3–H_9 and $H_{6\beta}$–H_9 strongly suggested that the carboxyl group at C_2 is below the C/D/E plane; the correlation between H_3 and $H_{5\beta}$ provided additional support for the β/β configuration of these two protons. Further evidence for the relative configuration of **1** came from the comparison of its ^{13}C NMR data with published data.[10,14,15] Although no report of the isolation of **1** from a natural source has appeared, it is interesting that a peracetylated derivative of **1** has been synthesized by a biomimetic route.[16] It may be noted that **1** could, in principle, have arisen by oxidative rearrangement[16,17] of vincosamide, either *in situ* or during storage of the extract prepared from the leaves of *A. javanicum*. In this context, however, it may be noted that no report has appeared documenting the presence of vincosamide itself in any plant from the genus *Alangium*.

In the presence of 20 μM Cu^{2+}, compound **1** exhibited moderate DNA strand scission activity‡ ranging from 10% conversion of supercoiled (Form I) DNA to nicked, circular (Form II) DNA at 10 μM **1** to 70% conversion at 200 μM **1**. It may be noted, however, that this is the first time that an oxindole alkaloid has been reported to cleave DNA, even though several oxindole-type alkaloids have been isolated from natural sources.[9,10,18]

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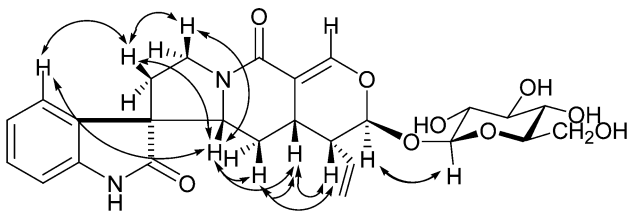

Fig. 3 NOEs from NOESY experiments involving javaniside.

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Notes and references

† Leaves of *A. javanicum* were collected in Sabah, Malaysia on October 17, 1987. A voucher specimen (Q6605281) is preserved at the U. S. National Arboretum Herbarium, Washington, DC, USA.

‡ The DNA cleavage assay was performed in 25 μl of 10 mM Tris–HCl (pH 8.0) containing 500 ng of supercoiled pBR322 plasmid DNA in the absence or presence of 20 μM $CuSO_4$ and crude extracts or fractions (dissolved in DMSO or 1:1 DMSO–MeOH to final concentrations of 100 and 50 μg ml^{-1}). Each experiment included controls containing DNA alone and DNA + $CuSO_4$. After incubation at 37 °C for 60 min, the reaction mixture was combined with 5.0 μl of 30% glycerol–0.01% Bromophenol Blue and was analyzed by electrophoresis in a 1.0% agarose gel containing 0.7 μg ml^{-1} ethidium bromide. Electrophoresis was carried out in 89 mM Tris–boric acid buffer, pH 8.3, containing 2 mM EDTA at 110–120 V for 2–3 h. Following electrophoresis, the gel was photographed under ultraviolet light.

1 See, for example: S. E. Sherman and S. J. Lippard, *Chem. Rev.*, 1987, **86**, 1153; I. H. Goldberg, L. S. Kappen, Y. J. Xu, A. Stassinopoulos, X. Zeng, Z. Xi and C. F. Yang, in *DNA and RNA Cleavers and Chemotherapy of Cancer and Viral Diseases*, ed. B. Meunier, NATO Advanced Sciences Institute Series, Series C, Kluwer Academic Publishers, Dordrecht, 1996, pp. 1–21.
2 E. C. Long, in *Bioorganic Chemistry: Nucleic Acids*, ed. S. M. Hecht, Oxford University Press, New York, 1996, pp. 3–35.
3 *Bleomycin Chemotherapy*, ed. B. I. Sikic, M. Rozencweig and S. K. Carter, Academic Press, Orlando, 1985; S. M. Hecht, in *Cancer Chemotherapeutic Agents*, ed. W. O. Foye, American Chemical Society, Washington, DC, 1995, p. 369ff.
4 R. T. Scannell, J. R. Barr, V. S. Murty, K. S. Reddy and S. M. Hecht, *J. Am. Chem. Soc.*, 1988, **110**, 3650; J. R. Barr, V. S. Murty, K. Yamaguchi, D. H. Smith and S. M. Hecht, *Chem. Res. Toxicol.*, 1988, **1**, 204; W. Lytollis, R. T. Scannell, H. An, V. S. Murty, K. S. Reddy, J. R. Barr and S. M. Hecht, *J. Am. Chem. Soc.*, 1995, **117**, 12 683; S. R. Starck, J.-Z. Deng and S. M. Hecht, *Biochemistry*, 2000, **39**, 2413.
5 L. A. Chrisey, G. H. S. Bonjar and S. M. Hecht, *J. Am. Chem. Soc.*, 1988, **110**, 644; S. K. Chaudhuri, L. Huang, F. Fullas, R. J. McGivney, D. M. Brown, M. C. Wani, M. E. Wall, J. C. Tucker, C. W. W. Beecher, J. M. Pezzuto and A. D. Kinghorn, *J. Nat. Prod.*, 1996, **56**, 290; L. Huang, M. E. Wall, M. C. Wani, H. Navarro, T. Santisuk, V. Reutrakul, E.-K. Seo, N. R. Farnesworth and A. D. Kinghorn, *J. Nat. Prod.*, 1998, **61**, 446; J.-Z. Deng, D. J. Newman and S. M. Hecht, *J. Nat. Prod.*, 2000, **63**, 1269.
6 T. Fazal, A. Rahman, J. Greensill, K. Ainky, S. M. Hudi and J. H. Parish, *Carcinogenesis*, 1990, **11**, 2005.
7 L. M. Weiner, *Methods Enzymol.*, 1994, **233**, 92; K. Fukuhara, M. Nagakawa and N. Miyata, *Abstr. Pap. - Am. Chem. Soc.*, 1998, **215**, 77.
8 J. Herscorici, C. Uriel, J. Uriel and K. Antonakis, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 421.
9 A. R. Araujo, C. Kascheres, F. Fujiwara and A. J. Marsaioli, *Phytochemistry*, 1984, **23**, 2359.
10 A. A. L. Gunatilaka, H. C. Fernando, A.-U. Rahman, M. M. Qureshi and S. Balasubramaniam, *Heterocycles*, 1989, **28**, 999.
11 A. Itoh, T. Tanahashi and N. Nagakura, *Chem. Pharm. Bull.*, 1994, **42**, 2208.
12 A. Itoh, T. Tanahashi and N. Nagakura, *Phytochemistry*, 1997, **46**, 1225.
13 A. Itoh, T. Tanahashi and N. Nagakura, *Heterocycles*, 1998, **48**, 499.
14 E. Wenkert, J. S. Bindra, C.-J. Chang, D. W. Cochran and F. M. Schell, *Acc. Chem. Res.*, 1974, **7**, 46.
15 A. Itoh, T. Tanahashi and N. Nagakura, *J. Nat. Prod.*, 1995, **58**, 1228.
16 A. Patthy-Lukáts, G. Beke, L. F. Szabó and B. Podnányi, *J. Nat. Prod.*, 2001, **64**, 1032.
17 G. A. Cordell, *Lloydia*, 1974, **37**, 219.
18 B. Weniger, Y.-L. Jiang, R. Anton, J. Bastida, T. Varea and J.-C. Quirion, *Phytochemistry*, 1993, **32**, 1587.