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Four alkaloids, lucidine B, oxolucidine A, lucidine A, and lucidulinone from *Lycopodium lucidulum*

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Abstract

The structures of four alkaloids extracted from *Lycopodium lucidulum* (Lycopodiaceae) were established by X-ray and 2D NMR spectroscopic analyses. The dihydro-derivative of oxolucidine A, which was obtained by $NaBH_4$ reduction of oxolucidine A, was treated with *p*-bromobenzoyl chloride to afford crystals, whose X-ray crystallographic analysis established the stereostructure, including the absolute configuration. The 2D NMR spectra of tetrahydrodeoxylucidine B were fully analyzed to establish the full structure of lucidine B, and the hitherto unknown stereochemistry at the C-14 position was established as β-H. The structure of a new alkaloid, lucidulinone, was determined by spectroscopic analysis to be luciduline lactam. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Lycopodium lucidulum; Lycopodiaceae; Alkaloids; Lucidine A; Lucidine B; Oxolucidine A; Oxolucidine B; Lucidulinone

1. Introduction

Lycopodium plants have long been studied and many alkaloids have been reported thus far (Ayer, Berezowsky & Law, 1963; Ayer, Masaki & Nkunika, 1968; Ayer, Altenkirk, Burnell & Moinas, 1969; Ayer & Altenkirk, 1969; Ayer, Browne, Nakahara, Tori & Delbaere, 1979; Ayer, Ball, Browne, Tori, Delbaere & Silverberg, 1984; Ayer & Trifonov, 1994). Most of the compounds reported have a common formula of $C_{10}N$ or $C_{16}N$ (Ayer & Trifonov, 1994) and representative compounds **1–17** including several specific to this study, are shown. Among them we have previously reported several $C_{30}N_3$ alkaloids (Ayer et al., 1979) including lucidine B (**1**) and lucidine A (**8**) and their congeners. Lucidine B (**1**) was correlated by chemical conversions (Ayer et al., 1979), and crystals of spirolucidine (**16**) were subjected to X-ray analysis (Ayer et al., 1984). However, the structures of the remaining alkaloids were too complicated to solve either by simple NMR spectroscopic techniques or by simple degradation procedures. Thus, further studies in this area were held in abeyance. Recently, huperzine was isolated from *Huperzia serrata* (= *L. serratum*) (Lycopodiaceae) as a potent inhibitor of acetylcholine esterase (Liu et al., 1986; Zhou et al., 1993). We also tested our sample collections of *Lycopodium* alkaloids for their anti-choline esterase activity, however, none were as active as huperzine (Liu et al., 1986; Ayer, Ma, Liu, Huang, Schultz & Clardy, 1994). This prompted a reinvestigation of the chemical constituents of the *L. lucidulum* extracts. As reported previously, lucidines A (**8**) and B (**1**) produce oxolucidines A (**9**) and B (**2**), respectively, on exposure to air (Ayer et al., 1979). This occurs due to the readily oxidizable imino group present in the structure (Cohen & Witkop, 1955). Furthermore, because of its acetamide moiety, its NMR spectrum is not easy to analyze at room temperature

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(Nkunika, 1967; Ball, 1971). Therefore, tetrahydrodeoxyoxolucidine B (**6**), which was derived from **4** by $LiAlH_4$ reduction, was converted to the dibenzoate **7**, and a crystal of which was used for X-ray analysis (Ayer et al., 1979). However, the stereochemistry at the C-14 position of lucidine B (**1**) itself was unknown. We now report the full structure of lucidine B (**1**) and oxolucidine A (**9**), as well as lucidulinone (**17**), based on X-ray and 2D-NMR spectroscopic analyses.

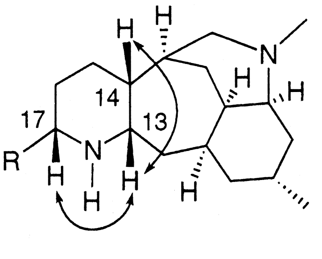


Fig. 2. NOEs around C-13 ~ C-17 for **5**

2. Results and discussion

Separation of lucidines A (**8**), B (**1**), oxolucidines A (**9**), B (**2**), and lucidulinone (**17**) was carried out by CC on alumina with different activities, followed by reversed-phase HPLC (Develosil ODS UG-5) using CH_3CN–H_2O (7:3). Lucidine B (**1**), $C_{30}H_{49}N_3O$, was treated with $LiAlH_4$ in THF under reflux to afford tetrahydrodeoxylucidine B (**5**), $C_{30}H_{53}N_3$, which was fully analyzed using ^{1}H-NMR spectroscope techniques. The molecular formula, $C_{30}H_{53}N_3$, was determined by HREIMS and the ^{13}C-NMR spectrum suggested the molecule lacked any oxygenated functionality. The ^{1}H-NMR spectrum showed the presence of an *N*-methyl group at δ2.11 (*s*) and ten protons adjacent to the nitrogen atoms, two of which were methylene protons of an *N*-ethyl group. Because the resonances of some protons were highly congested in $CDCl_3$, the NMR solvent was changed to C_6D_6. Its HSQC spectrum revealed the eight carbons next to the nitrogen atoms, one of which was the *N*-methyl group [δ_H 2.10(*s*); δ_C 44.0 (CH_3)]. The other characteristic peak was the methylene group of an *N*-ethyl group (C-1″) (δ_H 2.40 (*q*,$J = 7.1$ Hz); δ_C 48.5 (CH_2)). Long-range correlation peaks between the *N*-methyl group and two carbons (δ_C 68.2 (CH_2), 65.6 (CH)) attached to this nitrogen atom were also detected (Fig. 1). Therefore, the two carbons were assigned to C-2 and C-6, respectively. The assignment of C-2′ and C-6′ was carried out similarly by long-range correlations between the *N*-ethyl group and the methine (δ_C 54.5) and methylene (δ_C 46.2) carbons. The protons attached to these carbons were also assigned using the HSQC spectrum. Thus, six of the eight carbons adjacent to the nitrogen atoms were assigned, and the other two carbons should be C-13 (δ_C 59.2) and C-17 (δ_C 55.6).

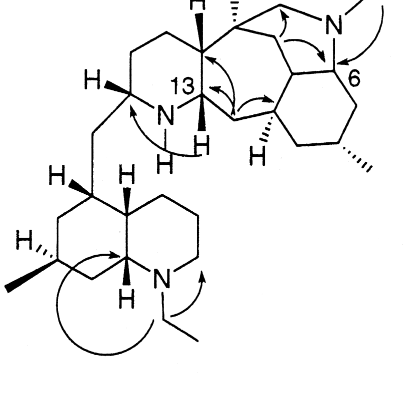


Fig. 1. Selected long-range correlations for **5**.

The proton at δ_H 3.03 (*br s*) attached to the carbon at δ_C 59.2 (C-13) showed a correlation with the carbon at δ_C 55.6 (C-17). Furthermore, the proton at δ_H 2.75 (*t*, $J = 13$ Hz), one of the methylene protons attached to δ_C 40.1 (C-11), had a correlation with the carbon at δ_C 59.2 (C-13). Because the carbon at δ_C 40.1 (C-11) could be correlated with C-10, 5, and 6, the resonance at δ_C 59.2 should be due to C-13, and that at δ_C 55.6 due to C-17. The other assignments were made by analysis of the COSY, HSQC, HMBC, and HSQC-TOCSY spectra. The NOESY spectrum indicated that H-13 and H-17 were on the same side of the molecule (Fig. 2). Moreover, H-13 also had a correlation peak with H-14. Therefore, these three protons are on the same side of the molecule. Since the rest of the configuration was verified by X-ray analysis of compound **7** (Ayer et al., 1979), the full structure of lucidine B (**1**) was established as depicted in the formula.

Oxolucidine A (**9**) was converted into dihydrooxolucidine A (**11**) $C_{30}H_{51}N_3O_2$, by treatment with $NaBH_4$ in MeOH. This in turn was treated with *p*-bromobenzoyl chloride with triethylamine in CH_2Cl_2 to afford **14** as crystals (mp 228–232°C) from MeOH. The ^{1}H-NMR spectrum of **14** showed the presence of more than two *p*-bromobenzoyl moieties and no acetamide group. The FABMS revealed the multiplet molecular ion peaks at *m*/*z* 1038, 1036, 1034, and 1032 indicating the presence of all three *p*-bromobenzoyl groups. Since it was not easy to analyze its NMR spectrum, X-ray analysis was performed next to yield a very unusual structure having two *p*-bromobenzoyl units at one *N*-acetamide position as well as one attached to N-18 (Fig. 3). This type of structure can be found in the literature (Kira, Zayed & Fathy, 1983), although it is unusual to obtain such a compound from a simple *p*-

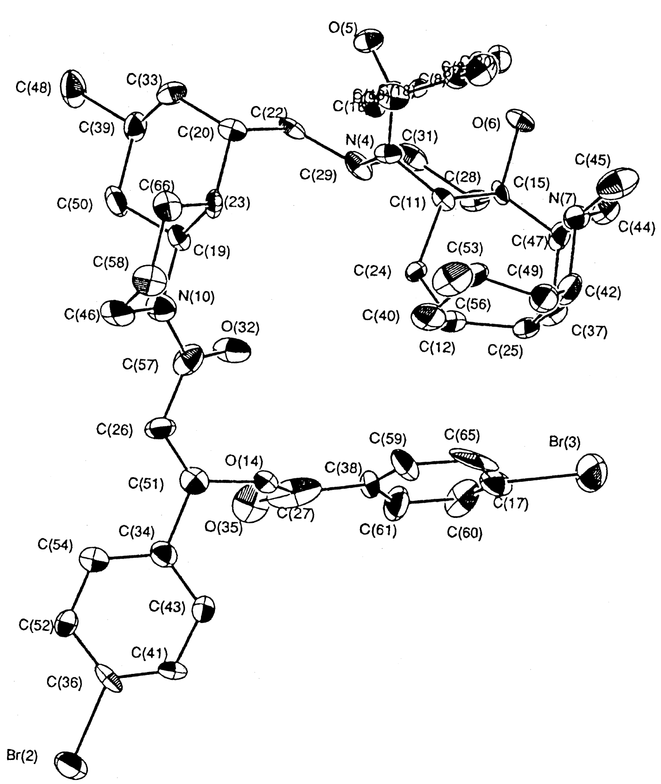


Fig. 3. ORTEP drawing of benzoate **14**.

bromobenzoylation reaction. In order to confirm this reaction, *N*,*N*-dimethylacetamide was treated with *p*-bromobenzoyl chloride to yield compound **15**. With the structure of oxolucidine A (**9**) established, it is apparent that the three chiral centers of **9** at the C-17, 5′, and 6′ positions differ from those of oxolucidine B (**2**). Because the η value was $+1.081$, the absolute configuration of **14** was also determined as depicted in the formula. Although we have speculated on the biogenesis of these compounds (Ayer et al., 1979), it is still necessary to accumulate examples of this type of alkaloids. It is interesting to note that the X-ray structure of compound **14** shows that the *cis*-decahydroquinoline rings adopt the chair forms similar to those of oxolucidine B (**2**) and spirolucidine (**16**). Spirolucidine (**16**) might be derived from oxolucidine A (**9**) by skeletal rearrangement, although attempted acid-catalyzed reaction of oxolucidine A (**9**) failed to produce spirolucidine (**16**) (Tori and Ayer, unpublished results).

Lucidulinone (**17**) displayed a molecular ion peak at *m*/*z* 222, indicating it to be a $C_{10}N$ type alkaloid. Its IR spectrum showed absorptions at 1720 and 1640 cm^{-1}, each revealing the presence of carbonyl and amide groups, respectively. The analysis of the HMBC spectrum showed correlations between the *N*-methyl group and the carbonyl and methine carbons. The secondary methyl group lies between the methylene carbons, as shown by the HMBC spectrum, and had COSY correlation peaks assignable to a $-CH-CH_2-CH(Me)-CH_2-CH-$ functionality. The degree of unsaturation of **17** was 5, indicating a tricyclic compound, and is therefore luciduline lactam, previously described in the literature (Ayer & Altenkirk, 1969; Szychowaki & MacLean, 1979), but isolated as a natural product for the first time.

From the weakly basic fractions lucidine A (**8**), oxolucidine A (**9**), lucidine B (**1**), oxolucidine B (**2**), and lucidulinone (**17**) were also isolated. The presence of these alkaloids was known for a long time, but the structures were unknown. This problem was now successfully solved by the combined use of 2D-NMR and X-ray crystallographic analyses. Although the acetylcholine esterase inhibition activities of these compounds were evaluated, no activity was observed (Tori, Shimoji, Takaoka, Nakashima, Sono & Ayer, 1999).

3. Experimental

3.1. General

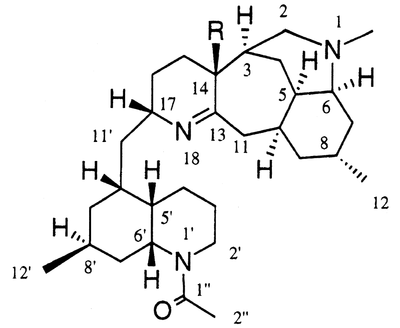
The IR spectra were obtained using with a JASCO FT/IR-5300 spectrophotometer. The ^{1}H-, ^{13}C-, and 2D NMR spectra were obtained using Varian Unity 600 (600 MHz), JEOL GX400 (400 MHz), or Varian Unity 200 (200 MHz) spectrometers. The mass spectra including high resolution mass spectra were recorded with a JEOL JMS AX-500 spectrometer. Develosil ODS-UG-5 was used for HPLC. Alumina N, Akt. I (ICN Biomedicals) was used for CC and aluminium oxide 60 F_{254} plates (Merck) for TLC.

3.2. Plant material

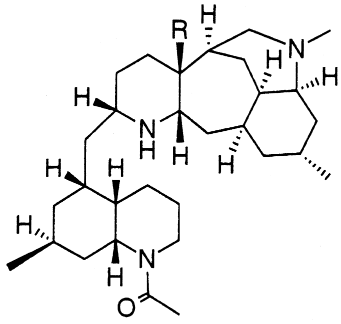
Lycopodium lucidulum (8.2 kg) was collected near Kesivick, York country, New Brunswick, Canada and was identified by W.A. Ayer. A voucher specimen is deposited in the herbarium of the University of Alberta.

3.3. Extraction and isolation

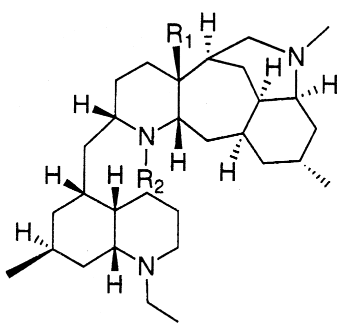
Pulverized *L. lucidulum* was extracted with MeOH to yield the methanol-soluble fraction (518 g). Acetone was added and the precipitate was removed. Acetone was then evaporated and the residue was digested with aqueous HCl (6%). The insoluble portion was filtered and then digested with aqueous HCl (10%). The precipitate was filtered off and the combined filtrates were washed with ether. The PH of the aqueous phase was adjusted to 10–11 with NH_4OH, and then extracted with $CHCl_3$. The organic layer was dried (K_2CO_3) and evaporated to afford the crude alkaloids (14 g). The total alkaloids were dissolved in aqueous HCl (10%)



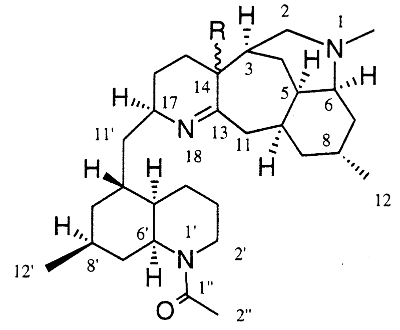
Lucidine B (**1**) R=H
Oxolucidine B (**2**) R=OH



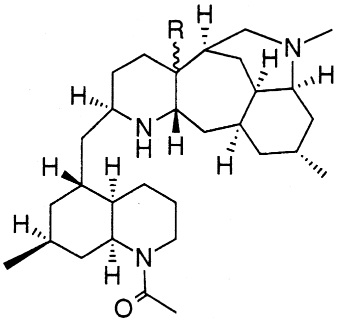
Dihydrolucidine B (**3**) R=H
Dihydrooxolucidine B (**4**) R=OH



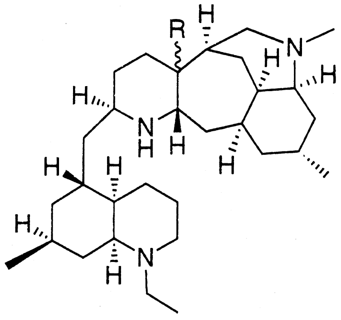
Tetrahydrodeoxylucidine B (**5**) $R_1=R_2=H$
Tetrahydrodeoxyoxolucidine B (**6**) $R_1=OH$, $R_2=H$
7 $R_1=R_2=OCOC_6H_4Br(p)$



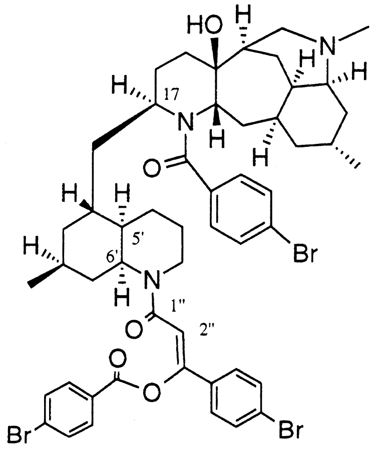
Lucidine A (**8**) R=H
Oxolucidine A (**9**) R=β–OH



Dihydrolucidine A (**10**) R=H
Dihydrooxolucidine A (**11**) R=β–OH



Tetrahydrodeoxylucidine A (**12**) R=H
Tetrahydrodeoxyoxolucidine A (**13**) R=β–OH



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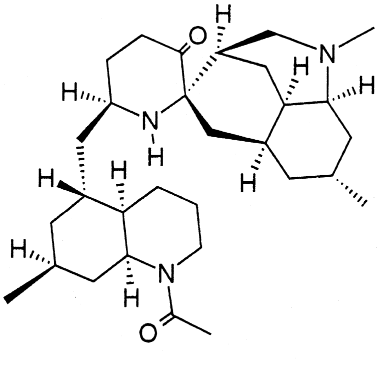
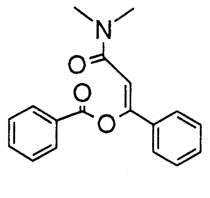


Spirolucidine (**16**)



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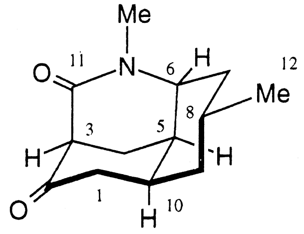

Lucidulinone (**17**)

and the pH of the solution was adjusted to 5.5 by addition of saturated Na_2HPO_4. Extraction with $CHCl_3$ afforded the weak bases (10 g). The pH of the solution was then raised to 10–11 by the addition of NH_4OH. Extraction with $CHCl_3$ afforded the strong bases (3.2 g). The weak bases (10 g) were subjected to a 10-funnel counter current distribution between $CHCl_3$ and saturated potassium hydrogen tartrate solution to afford three frs. The weaker bases fr. A (987 mg) were separated by CC on alumina followed by reversed phase HPLC (Develosil ODS-UG-5; elution with CH_3CN–H_2O, 7:3) to yield lucidine A (**8**) (34 mg), lucidine B (**1**) (108 mg), oxolucidine A (**9**) (401 mg), oxolucidine B (**2**) (181 mg), and lucidulinone (**17**) (27.6 mg).

Lucidine A (**8**): EIMS (rel. int.) *m*/*z* 467 $[M]^+$ (22), 424 (5), 273 (100), 260 (15), 217 (10), 164 (9); FTIR ν_{max} cm^{-1}: 2800, 1640, 1460, 1280, 760.

Lucidine B (**1**): EIMS (rel. int.) *m*/*z* 467 $[M]^+$ (49), 455 (25), 424 (12), 289 (23), 273 (100), 260 (87), 248 (20), 233 (56), 218 (34), 205 (28), 164 (43); FTIR ν_{max} cm^{-1}: 2800, 1660, 1460, 1450, 1390, 1280, 760.

Oxolucidine A (**9**): $[\alpha]_D^{21.5}$ − 29.1° ($CHCl_3$, *c* 0.74); HREIMS *m*/*z* found 483.3817. Calcd for $C_{30}H_{49}O_2N_3$ 483.3826; EIMS (rel. int.) *m*/*z*: 483 $[M]^+$ (46), 465 (59), 455 (100), 426 (19), 398 (7), 289 (47), 271 (82), 233 (56), 205 (84), 192 (51); FTIR ν_{max} cm^{-1}: 3400, 2800, 1640, 1460, 1280, 760.

Oxolucidine B (**2**): $[\alpha]_D^{21.5}$ − 30.7° ($CHCl_3$, *c* 0.55); HREIMS *m*/*z* found 483.3846. Calcd for $C_{30}H_{49}N_3O_2$ 483.3825; EIMS (rel. int.) *m*/*z* 483 $[M]^+$ (82), 465 (42), 455 (47), 440 (8), 398 (10), 289 (44), 276 (100), 233 (93), 205 (34), 164 (39); FTIR ν_{max} cm^{-1}: 3400, 1640, 1460, 1280, 760.

Lucidulinone (**17**): $[\alpha]_D^{21.5}$ + 185.0°C ($CHCl_3$, *c* 1.1); HRCIMS *m*/*z* found *m*/*z* 222.1491 $[M + H]^+$ Calcd. for $C_{13}H_{19}O_2N$ 222.1494; CIMS (rel. int.) *m*/*z* 222 $[M + H]^+$ (100), 193(5), 165 (5); FTIR ν_{max} cm^{-1}: 1720, 1640; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 3.67 (1H, *q*, $J =$ 3.3 Hz, H-6), 3.33 (1H, *q*, $J =$ 1.9 Hz, H-3), 2.99 (3H, *s*, H-13), 2.63 (1H, *dd*, $J =$ 14.8, 12.4 Hz, H-1β), 2.38 (1H, *m*, H-10), 2.33 (1H, *dt*, $J =$ 14.3, 3.3 Hz, H-4β), 2.27 (1H, *m*, H-7β), 2.25 (1H, *m*, H-1α), 2.10 (1H, *m*, H-5), 2.05 (1H, *m*, H-4α), 1.70 (1H, *m*, H-8β), 1.58 (1H, *dq*, $J =$ 13.5, 2.3 Hz, H-9β), 1.38 (1H, *ddd*, $J =$ 13.5, 12.1, 4.6 Hz, H-9α), 1.25 (1H, *m*, H-7α), 0.97 (3H, *d*, $J =$ 6.6 Hz, H-12); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 205.9 (C-12), 167.6 (C-2), 58.2 (C-6), 55.9 (C-3), 42.7 (C-11), 38.9 (C-9), 38.1 (C-7), 36.4 (C-10), 33.1 (C-5), 31.3 (C-4), 30.4 (C-14), 21.8 (C-13), 20.2 (C-8); Δε (298 nm) +7.0 ($CHCl_3$).

*3.3.1. Preparation of tetrahydrodeoxylucidine B (**5**)*

A solution of lucidine B (**1**) (68 mg) in dry THF (8 ml) was treated with $LiAlH_4$ (100 mg) under reflux overnight. Ethyl acetate, water (0.1 ml), 15% NaOH (0.1 ml), and water (0.3 ml) were added successively. The precipitate was removed and the solvent was evaporated to afford a residue (59 mg), which was purified by CC on alumina (benzene–EtOAc, in gradient) to give tetrahydrodeoxylucidine B (**5**) (30 mg); HREIMS *m*/*z* found 455.4245. Calcd. for $C_{30}H_{53}N_3$ 455.4240; EIMS (rel. int.) *m*/*z* 455 $[M]^+$ (100), 440 (12), 426 (19), 398 (19), 384 (8), 343 (18), 301 (16), 275 (47), 261 (99), 180 (43); ^{1}H-NMR (600 MHz, C_6D_6) δ 3.18 (1H, *d*, $J =$ 12 Hz, H-4α), 3.10 (1H, *dt*, $J =$ 13, 4 Hz, H-6′), 3.03 (1H, *br s*, H-13), 2.75 (1H, *t*, $J =$ 13 Hz, H-11β), 2.68 (1H, *m*, H-17), 2.57 (1H, *br d*, $J =$ 10 Hz, H-2′β), 2.47 (1H, *d*, $J =$ 10.7 Hz, H-2β), 2.40 (2 H, *q*, $J =$ 7.1 Hz, H-1″), 2.26 (1H, *br t*, $J =$ 10 Hz, H-2′α), 2.22 (1H, *m*, H-10), 2.10 (3H, *s*, N-Me), 2.08 (1H, *m*, H-7), 2.06 (1H, *m*, H-5′), 2.05 (1H, *m*, H-2α), 2.03 (1H, *m*, H-8′), 1.96 (1H, *m*, H-10′), 1.93 (1H, *m*, H-8), 1.90 (1H, *q*, $J =$ 3 Hz, H-6), 1.70 (1H, *br s*, H-14), 1.66 (1H, *br s*, H-5), 1.65 (1H, *m*, H-3′), 1.62 (2 H, *m*, H-3, 7′), 1.61 (1H, *m*, H-16), 1.59 (1H, *m*, H-16), 1.58 (1H, *m*, H-11α), 1.54 (1H, *m*, H-3′), 1.53 (1H, *m*, H-9), 1.50 (1H, *m*, H-4β), 1.45 (1H, *m*, H-15), 1.38 (2H, *m*, H-4′, 11′), 1.32 (1H, *m*, H-9′), 1.28 (1H, *m*, H-11′), 1.26 (1H, *m*, H-4′), 1.22 (2H, *m*, H-9, 9′), 1.20 (1H, *m*, H-15), 1.14 (1H, *m*, H-7′), 1.08 (3H, *t*, $J =$ 7.1 Hz, H-2″), 0.98 (3H, *d*, $J =$ 7.3 Hz, H-12′), 0.88 (1H, *m*, H-7), 0.88 (3H, *d*, $J =$ 6.6 Hz, H-12); ^{13}C-NMR (150 MHz, C_6D_6) 68.2 (C-2), 65.6 (C-6),δ 59.2 (C-13), 55.6 (C-17), 54.5 (C-6′), 48.5 (C-1″), 46.2 (C-2′), 44.0 (N-Me), 43.9 (C-9), 43.6 (C-14), 41.8 (C-11′), 40.4 (C-5′), 40.1 (C-3, 11), 39.5 (C-5, 7), 36.1 (C-16), 34.0 (C-9′), 33.2 (C-10), 31.7 (C-4), 30.9 (C-10′), 28.4 (C-15), 27.9 (C-8′), 25.8 (C-3′), 23.2 (C-12), 21.9 (C-8), 21.7 (C-7′), 19.1 (C-12′), 17.8 (C-4′), 13.3 (C-2″).

*3.3.2. Preparation of tetrahydrodeoxyoxolucidine B (**6**)*

A solution of oxolucidine B (**2**) (115 mg) in dry THF (20 ml) was treated with $LiAlH_4$ (250 mg) under reflux for 24 h. Wet Et_2O, EtOAc, and 1 M HCl solution were added and the mixture was extracted with Et_2O. The aqueous solution was basified with NH_4OH to pH 12 and the mixture was extracted with $CHCl_3$. The organic solution was washed with brine, dried ($MgSO_4$), and evaporated to afford tetrahydrodeoxyoxolucidine B (**6**) (81 mg); HREIMS *m*/*z* found 471.4195. Calcd. for $C_{30}H_{53}N_3O$ 471.4189; EIMS (rel. int.) *m*/*z* 471 $[M]^+$ (10), 453 (100), 438 (9), 409 (6), 396 (7), 289 (10), 259 (28), 227 (7), 192 (27), 164 (22); FTIR ν_{max} cm^{-1}: 3410, 1460, 1390, 740; ^{1}H-NMR (600 MHz, C_6D_6) δ 0.81 (3H, *d*, $J =$ 6.3 Hz, H-12), 0.99 (3H, *d*, $J =$ 7.6 Hz, H-12′), 1.09 (3H, *t*, $J =$ 7.1 Hz, H-2″), 1.54 (1H, *m*, H-11′), 1.57 (2H, *m*, H-5, 3′), 1.60 (1H, *m*, H-3′), 1.68 (2H, *m*, H-8, 7′), 1.74 (1H, *dd*,$J =$ 8.5, 2.4 Hz, H-2), 1.80 (1H, *dd*, $J =$ 10, 3.9 Hz, H-15), 1.82 (3H, *m*, H-3, 6, 15), 1.89 (2H, *m*, H-7, 10′), 1.91 (3H, *s*, N-Me), 2.01 (1H, *m*, H-8′), 2.02 (1H, *m*, H-5′), 2.10 (1H, *m*, H-10), 2.31 (1H, *td*, $J =$ 9.3, 2.9

Hz, H-2′), 2.42 (2H, *m*, H-1″), 2.57 (1H, *m*, H-2′), 2.58 (1H, *t*, $J = 13.2$ Hz, H-11), 2.75 (1H, *m*, H-17) 2.93 (1H, *d*, $J = 5.1$ Hz, H-13), 3.08 (1H, *dt*, $J = 12.9$, 3.8 Hz, H-6′), 3.26 (1H, *dt*, $J = 9.9$, 2.4 Hz, H-2), 3.47 (1H, *br d*, $J = 11.4$ Hz, H-4); ^{13}C-NMR (150 MHz, C_6D_6) δ 13.6 (C-2″), 18.0 (C-4′), 19.2 (C-12′), 21.5 (C-8, 7′), 23.0 (C-12), 26.1 (C-3′), 27.9 (C-8′), 30.1 (C-11′), 31.2 (C-4), 31.3 (C-10′), 33.2 (C-10), 34.0 (C-9′), 34.4 (C-11), 38.7 (C-7), 39.2 (C-5), 40.8 (C-5′), 41.3 (C-16), 42.6 (C-3), 43.2 (C-15, N-Me), 43.4 (C-9), 46.1 (C-2′), 48.5 (C-1c″), 54.5 (C-6′), 55.2 (C-17), 62.2 (C-2), 65.7 (C-6), 67.7 (C-13), 71.9 (C-14).

3.3.3. Preparation of the di-p-bromobenzoate of tetrahydrodeoxyoxolucidine B (7)

A solution of tetrahydrodeoxyoxolucidine B (**6**) (23.9 mg) in dry pyridine (1 ml) was treated with *p*-bromobenzoyl chloride (112 mg) at room temperature for 2 days. Water was added and the mixture was extracted with $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and was evaporated to give a residue (73.4 mg), which was purified by CC on alumina (grade III) elution with a hexane–EtOAc gradient afforded the benzoate **7** (15 mg); $[\alpha]_D^{21.5}$ − 30.5° ($CHCl_3$, *c* 0.89); HRFABMS *m*/*z* found 836.2978 [M + H]$^+$. Calcd. for $C_{44}H_{60}N_3O_3Br_2$ 836.300; FABMS (rel. int.) *m*/*z* 840 (21), 838 (44), 836 [M + H]$^+$ (37), 636 (67), 473 (58), 452 (37), 180 (100); FTIR ν_{max} cm^{-1}: 3450, 2950, 2790, 1710, 1630; λ_{max} 243 nm (ε 16,000); $\Delta\varepsilon$ (249 nm) − 12.1 ($CHCl_3$).

3.3.4. Preparation of dihydrooxolucidine A (11)

A solution of oxolucidine A (**9**) (47 mg) in MeOH (1 ml) was treated with $NaBH_4$ (30 mg) at room temperature for 2 h. Water was added and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated to give dihydrooxolucidine A (**11**) (66 mg); $[\alpha]_D^{21.5^\circ}$ +3.9° ($CHCl_3$, *c* 0.79); HRMS *m*/*z* found 485.3903. Calcd. for $C_{30}H_{51}N_3O_2$ 485.3903; EIMS (rel. int.) *m*/*z* 485 [M]$^+$ (4), 467 (100), 452 (14), 424 (8), 410 (4), 291 (8), 273 (43), 259 (39), 235 (8), 192 (9), 163 (15); FTIR ν_{max} cm^{-1}: 3400, 2925, 2860, 2775, 1635, 1620, 750; ^{13}C-NMR (150 MHz, $CDCl_3$) δ 168.9, 168.8, 72.5, 58.6, 58.5, 52.8, 46.9, 43.2, 41.9, 39.9, 38.5, 38.4, 38.3, 36.5, 34.5, 33.8, 33.6, 32.4, 27.1, 26.9, 26.5, 25.8, 25.7, 25.6, 22.9, 22.6, 22.5, 22.3, 21.6, 21.5.

3.3.5. Preparation of p-bromobenzoyl derivative of dihydrooxolucidine A (14)

A solution of dihydrooxolucidine A (**11**) (66 mg) in dry CH_2Cl_2 (2 ml) was treated with *p*-bromobenzoyl chloride (300 mg), Et_3N (0.2 ml), and DMAP (5 mg) at room temperature for 24 h. Water was added and the residue was extracted with $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated to give a residue (169 mg). The residue was purified by CC on alumina (grade III) eluting with a hexane–EtOAc gradient to afford pure **14** (22.5 mg); *p*-bromobenzoate (**14**); mp 228–232°C (from MeOH); $[\alpha]_D^{21.5^\circ}$ + 7.3° ($CHCl_3$, *c* 1.1); HRFABMS *m*/*z* found 1032.2150 [M + H]$^+$. Calcd. for $C_{51}H_{61}Br_3N_3O_5$ 1032.2155; FABMS (rel. int.) *m*/*z* 1038 (17), 1036 (39), 1034 (40), 1032 [M + H]$^+$ (12), 1017 (15), 850 (6), 624 (10), 307 (12), 183 (100), 154 (62), 136 (48); FTIR ν_{max} cm^{-1}: 3450, 2925, 2860, 2775, 1740, 1625; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 0.75 (3H, *d*, $J = 6.32$ Hz), 1.24 (3H, *d*, $J = 7.14$ Hz), 1.25 (3H, *s*), 6.42 (*s*) and 6.47 (*s*).

*3.4. X-ray analysis of **14***

Reflection data were obtained with a Mac Science MXC 18 diffractometer using copper radiation Cu Kα ($\lambda = 1.54178$ Å). All diagrams and calculations were performed using CRYSTAN.

Crystal dimension = 0.5 × 0.2 × 0.05 mm, monoclinic space group P_{21}, $a = 18.415$ (6) A, $b = 9.190$ (7) A, $c = 15.002$ (2) A, $v = 2462.419922(0)$ A3, Z = 2, $D_{calc} = 1.128$, 1568 observed reflections, $S = 2.565$, final residuals R and R_w with 0.056, 0.067, $Eta = +1.08133$. The data have been deposited at the Cambridge Crystallographic Data Centre (Tori et al., 1999).

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References

Ayer, W. A., Berezowsky, J. A., & Law, D. A. (1963). *Can. J. Chem*, *41*, 649–657.

Ayer, W. A., Masaki, N., & Nkunika, D. S. (1968). *Can. J. Chem*, *46*, 3631–3642.

Ayer, W. A., Altenkirk, B., Burnell, R. H., & Moinas, M. (1969). *Can. J. Chem*, *47*, 449–455.

Ayer, W. A., & Altenkirk, B. (1969). *Can. J. Chem*, *47*, 499–502.

Ayer, W. A., Browne, L. M., Nakahara, Y., Tori, M., & Delbaere, L. T. J. (1979). *Can. J. Chem*, *57*, 1105–1107.

Ayer, W. A., Ball, L. F., Browne, L. M., Tori, M., Delbaere, L. T. J., & Silverberg, A. (1984). *Can. J. Chem*, *62*, 298–302.

Ayer, W. A., & Trifonov, L. S. (1994). Lycopodium alkaloids. In *The Alkaloids* (pp. 233–2625). New York: Academic Press.

Ayer, W. A., Ma, Y. T., Liu, J. S., Huang, M. F., Schultz, L. W., & Clardy, J. (1994). *Can. J. Chem*, *72*, 128–130.

Liu, J. S., Zhu, Y. L., Yu, C. M., Zhou, Y. Z., Han, Y. Y., Wu, F. W., & Qui, B. F. (1986). *Can. J. Chem*, *64*, 837–839.

Ball, L. F. (1971). Ph.D. Thesis, University of Alberta, Edmonton, Alberta.

Cohen, L. A., & Witkop, B. (1955). *J. Am. Chem. Soc*, *77*, 6595–6600.

Kira, M. A., Zayed, A. A., & Fathy, N. M. (1983). *Egypt. J. Chem*, *26*, 253–254.

Nkunika, D. S. (1967). Ph.D. Thesis, University of Alberta, Edmonton, Alberta.

Szychowaki, J., & MacLean, D. B. (1979). *Can. J. Chem*, *57*, 1631–1637.

Tori, M., Shimoji, T., Takaoka, S., Nakashima, K., Sono, M., & Ayer, W. A. (1999). *Tetrahedron Lett*, *40*, 323–324.

Zhou, B. N., Zhu, D. Y., Huang, M. F., Lin, L. J., Lin, L. Z., Han, X. Y., & Cordell, G. A. (1993). *Phytochemistry*, *34*, 1425–1428.