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Heteroyohimbinoid type oxindole alkaloids from *Mitragyna parvifolia*

Richa Pandey, Subhash C. Singh, Madan M. Gupta *

Central Institute of Medicinal and Aromatic Plants, Analytical Chemistry Division, P.O. CIMAP, Kukrail Picnic Spot Road, Lucknow, Uttar Pradesh 226015, India

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Abstract

The leaves of the plant *Mitragyna parvifolia* have afforded two alkaloids, 16,17-dihydro-17β-hydroxy isomitraphylline (**1**) and 16, 17-dihydro-17β-hydroxy mitraphylline (**2**), together with two known alkaloids, isomitraphylline (**3**) and mitraphylline (**4**). The structures of **1** and **2** were elucidated using 1D and 2D NMR spectral methods, including ^{1}H-^{1}HCOSY, DEPT, HSQC, and HMBC. Mitraphylline was the main alkaloid constituent.

Keywords: *Mitragyna parvifolia*; Rubiaceae; Alkaloids; Mitraphylline

1. Introduction

The genus *Mitragyna* (family: Rubiaceae) consists of trees growing exclusively in humid conditions. Several of these plants have been used in local folklore medicine for a wide variety of diseases such as fever, colic, muscular pains and for the expulsion of worms (Shellard and Phillipson, 1964) and commercially in the timber and paper industry (Chatterjee et al., 1982). Shellard and his co-workers (Shellard et al., 1969a; Shellard and Houghton, 1971, 1974) have contributed significantly towards the chemotaxonomic studies of this genus. *Mitragyna parvifolia* (Roxb.) Korth is a much-investigated species. It has been observed that environmental factor has a vital role to play in modifying the alkaloid content and the structure (Chatterjee et al., 1982; Avadhoot and Varma, 1991). Though various indolic and oxindolic alkaloids have been reported from the species only six alkaloids, all oxindolic, viz. mitraphylline, isomitraphylline, pteropodine, isopteropodine, speciophylline and uncarine F have been reported from the Lucknow region (Shellard et al., 1969b). As these alkaloids are of significant biological importance (Stuppner et al., 1993) it was thought worthwhile to study the alkaloidal profile of *M. parvifolia* growing in Lucknow. This paper describes the isolation and structure elucidation of two new alkaloids in addition to known alkaloids, viz., isomitraphylline (**3**), and mitraphylline (**4**).

2. Results and discussion

A total of four heteroyohimbine type oxindole alkaloids were isolated from an acid base treated chloroform fraction of ethanolic extract of *M. parvifolia* leaves. Compounds **3** and **4** were identified as isomitraphylline and mitraphylline, respectively, from their spectroscopic data and by comparison of the data with the literature (Seki et al., 1993). The known mitraphylline (**4**) was found to be the main constituent in the extract. On the basis of their similar spectral data isolates, **1** and **2**, were assumed to have structures closely related to **3** and **4**, respectively. Complete and unambiguous ^{1}H and ^{13}C NMR assignments were possible for the isolates **1** and **2**, with the use of ^{1}H-H correlated spectroscopy (COSY), heteronuclear single quantum coherence experiment via direct coupling (HSQC) and heteronuclear multiple bond correlation spectrum (HMBC). The DEPT

* Corresponding author. Tel.: +91 522 2359632; fax: +91 522 2342666.
E-mail address: guptammg@rediffmail.com (M.M. Gupta).

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experiment was used to ascertain the number of sp, sp^2, sp^3, and quaternary carbon atoms.

In its FAB-mass spectrum, isolate **1** showed a molecular ion peak at *m/z* 387 $[M + H]^+$, 18 mass units more than that of isomitraphylline (**3**). Ion peak at *m/z* 369 $[M + H–H_2O]^+$ resulted due to loss of a water molecule. The molecular formula of isolate **1** was found to be $C_{21}H_{26}N_2O_5$ from its FAB MS and NMR data. The infrared absorption spectrum showed bands at ν_{max} 3423 and 3250 cm^{-1} indicative of imino and hydroxyl groups in the molecule. The bands at 1720 and 1705 cm^{-1} were attributable to an ester carbonyl and an oxindole carbonyl, respectively (Seaton et al., 1958). The bands at 1622 and 755 cm^{-1} were due to a benzene ring, and at 1102 cm^{-1} was indicative of a cyclic ether (Ban et al., 1976). Barring a few signals the ^1H and ^{13}C NMR spectrum of **1** bear a close resemblance to that of **3**. Signals for the ring A, B, and C, did not show any significant shift in the absorption positions in both the spectra. The most notable difference between **3** and **1** was the disappearance of the olefinic signal at δ 7.37 (H-17) present in the ^1H NMR spectra of **3** from that of **1**. Instead **1** showed new additional absorption signals at δ 4.99 (1H, *d*, $J = 8.4$ Hz) and 2.24 (1H, *m*) correlating with carbon signals at δ 91.2 and 56.6, respectively, in its HSQC spectrum. Similar to **3** the ^1H NMR spectra of **1** showed the four proton signals in its lowfield region at δ 6.87 (1H, *d*, $J = 7.6$ Hz, H-12), 6.97 (1H, *ddd*, $J = 7.6$, 7.6, 0.8 Hz, H-10), 7.14 (1H, *ddd*, $J = 7.6$, 7.6, 1.2 Hz, H-11), and 7.31 (1H, *d*, $J = 7.6$ Hz, H-9) correlating with signals at δ 109.9, 122.9, 128.1, and 125.3, in the ^{13}C NMR spectra, as determined by the HSQC experiment, due to an indole nucleus. Thus, it confirmed that the oxindole moiety is unsubstituted in the aromatic ring (Shellard et al., 1971). The HSQC experiment revealed that there were two pairs of signals for nonequivalent methylene gem protons, viz., δ 0.69, 2.21, and δ 1.92, 3.11. The former gem protons had connectivity with a signal at δ 2.52 (1H, *dd*, $J = 11.5$, 3.2 Hz) which correlated with the carbon signal at δ 71.2 in the HSQC experiment. Therefore, the proton at δ 2.52 had to be assigned as H-3 since this was the only position at which a proton could have coupling with an adjacent methylene group (C-14). Of the C-14 methylene protons, the one showing a large upfield shift at δ 0.69, could be assigned as 14β-H from the observation of the large coupling constants with 15H ($J = 11.5$ Hz) and 3H ($J = 11.5$ Hz). The coupling constant between one of the C-21 protons at δ 1.92 and H-20 was 11.0 Hz, a typical axial–axial coupling constant. Therefore, the signal at δ 1.92 (1H, *dd*, $J = 11.0$, 11.0 Hz) was assigned to the α-oriented proton at the C-21 position. The geminal pair at δ 2.54 (1H, *ddd*, $J = 8.9$, 8.9, 8.9 Hz) and 3.30 (1H, *ddd*, $J = 8.9$, 8.0, 2.1 Hz) was correlated with the carbon signal at δ 53.9 while the geminal pair at δ 2.41 (1H, *ddd*, $J = 13.0$, 8.9, 2.3 Hz) and 2.04 (1H, *ddd*, $J = 13.0$, 8.9, 8.6 Hz) correlated with the carbon signal at δ 35.7 in the HSQC spectrum. The protons of C-21, C-6, and C-3, showed connectivity to the signal at δ 53.9 in the HMBC experiment, while the protons of C-3, and C-5, showed correlation in the HMBC spectra with the carbon signal at δ 35.7. The proton signal at δ 2.41 also showed connectivity with the carbonyl carbon δ 181.7 (C-2). Therefore, the former nonequivalent proton containing methylene group was assigned the position C-5 and the latter at C-6. Thus, the ^1H and ^{13}C NMR signals for the rings A, B, C, and D, of **1** were nearly identical to that of **3**. Departure from such similarity was noticed in the signals of ring E. The three proton doublet at δ 1.17 in the ^1H NMR spectrum of **1** is indicative of a closed E ring and a trans D/E ring junction similar to **3**, as in the case of seco E ring a three proton triplet is shown at δ 0.86 for the C-18 Me group (Shellard et al., 1967, 1971). The olefinic bond between C-16 and C-17 in **3** has been hydrogenated in **1** which has an extra hydroxyl group, as is evident from the FABMS and IR spectrum. The only position free in ring E for the introduction of a hydroxy group is C-17. This assumption was proved by comparing the shifts observed for ring E in both the NMR spectrum with those of ajmalicinine (**5**), a heteroyohimbine type indole alkaloid having a hydroxyl group at C-17 (Madinaveitia et al., 1996). Similar to **5** the oxygenated signal at δ 4.14 (1H, *qd*, $J = 6.8$, 3.7 Hz) was shown to be of C-19 position as it showed coupling to the protons of C-18 and C-20 in the ^1H-H COSY spectrum. The signal at δ 4.99 (1H, *d*, $J = 8.4$ Hz) was assigned to C-17 as it showed connectivity to the ester carbonyl and C-16 proton in the HMBC spectra. Comparison of the ^1H NMR shifts observed in the H-17β equatorial and H-17α axial protons in the H-17 anomers of ajmalicinine made possible the assignment of H-17 in **1** as equatorial as in the case of axial proton the signal appears more downfield at δ 5.50 (1H, *d*, $J = 3.5$ Hz) with a smaller coupling constant. The H-16 proton appeared at δ 2.24 as a multiplet. The assignments of the quaternary carbons in **1** were made by means of HMBC experiment. One of the two carbonyl carbon at δ 181.7 had a long range correlation with protons of C-6 and C-3 showing that this was the amide carbonyl carbon at the C-2 position. The other carbonyl carbon at δ 172.6 had connectivity with H-17 at δ 4.99. So this was the ester carbonyl carbon. The observation of long range coupling between H-6 with δ 133.7 enabled the distinction between C-8 and C-13. The correlation shown between protons of C-5 and C-6 with a signal at δ 56.8 confirmed its position at C-7 (See Fig. 1).

The stereochemistry of the compound **1** at different stereogenic centre was assigned by comparison of chemical shifts as reported for different stereoisomers by Seki et al. (1993) for heteroyohimbinoid type oxindole alkaloids. As in the ^1H NMR spectra of **1**, the signals of H-15 appeared at δ 1.95, it belonged to the *normal*-type (3*S*, 20*R* with *trans* D/E ring relationship) as in the case of *normal* type H-15 appears in the range δ 2.0–2.2 while in case of *allo* type and *epiallo* type it resonates between δ 2.4–2.5, and δ 2.7–2.9, respectively. Also, the coupling constant between H-15 and H-14β was large ($J = 11.5$ Hz) as is the case of *normal* type ($J = 10$–11 Hz) groups. The C/D ring junction

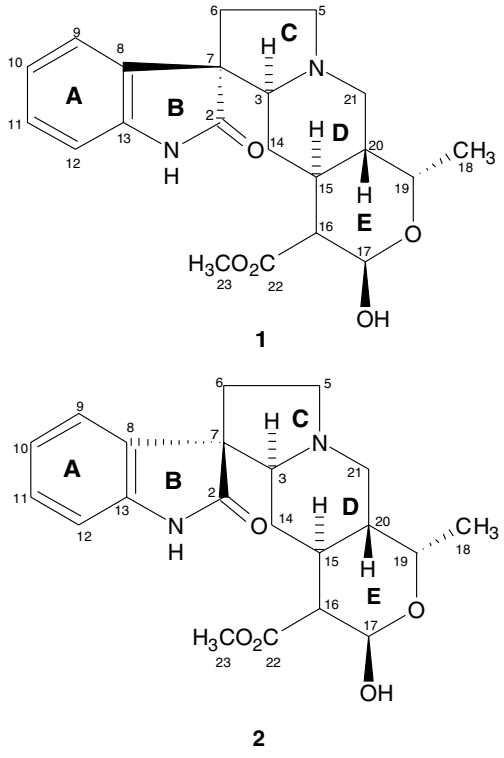

Fig. 1. Structures of compounds **1** and **2**.

was determined to be *trans* as is the case in normal type oxindole alkaloids (Shellard et al., 1969b) with the help of CD spectra (Fig. 2) which was similar to isomitraphylline (**3**) (Poisson and Pousset, 1967). The stereochemistry at stereocenter (C-7) was determined on the basis of chemical shifts of protons and carbons at the C-3 and C-9 positions. The 3-H signal appeared at δ 2.52 which is within the range of 7(*S*) isomers (δ 2.5–2.6) but it shifts upfield in the case of 7(*R*) isomers at δ 2.3–2.4. The H-9 signals appear at δ 7.3–7.35 in the 7(*S*) isomers and at around δ 7.2 in the 7(*R*) isomers for the *normal* type compounds. In the case of **1**, the H-9 signal appeared at δ 7.31, indicating the 7(*S*) stereochemistry. Moreover, the carbon signals were also in accordance with 7(*S*) configuration appearing at δ 71.2 for C-3 (range between δ 71.2–71.8 for *S*-isomers) which in case of 7(*R*) isomer resonates at δ 74.0–74.5. The stereochemistry at C-19 was determined on the basis of coupling constant between 19-H and 20-H. In 19(*S*) $J = 3$–4 Hz (*cis*) while in 19(*R*), $J = 10$–12 Hz (*trans-diaxial*). As in **1** the coupling constant was found to be 3.7 Hz, it has 19(*S*) configuration. The ^{13}C NMR substantiated this as the C-15 signals of the normal type compounds are observed at δ 30–30.5 in the 19(*S*) isomer and at δ 36–36.5 in 19(*R*) isomer. Though in **1** there was a downfield shift in C-15 position that can be explained due to the presence of hydroxyl group in place of olefinic bond, still the absorption position was not in the 19(*R*) isomer range.

On acetylation **1** afforded a monoacetate **1a**. The signal due to H-17 appeared at δ 6.46 showing a downfield shift.

Therefore, on the basis of above spectral data the structure of **1** can be postulated as 16,17-dihydro-17β-hydroxyisomitraphylline having 3*S*, 4*R*, 7*S*, 15*S*, 19*S*, 20*R*, configuration.

The FABMS of isolate **2** also showed a molecular ion peak at *m/z* 387 $[M + H]^+$, indicating it to be isomeric with **1**. Loss of a molecule of water resulted in a mass peak at *m/z* 369 $[M + H–H_2O]^+$. The molecular formula of isolate **2** was deduced as $C_{21}H_{26}N_2O_5$on the basis of FABMS and NMR data. The IR spectrum was also similar to **1** showing absorption bands at ν_{max} 3371 and 3254 cm^{-1} due to an

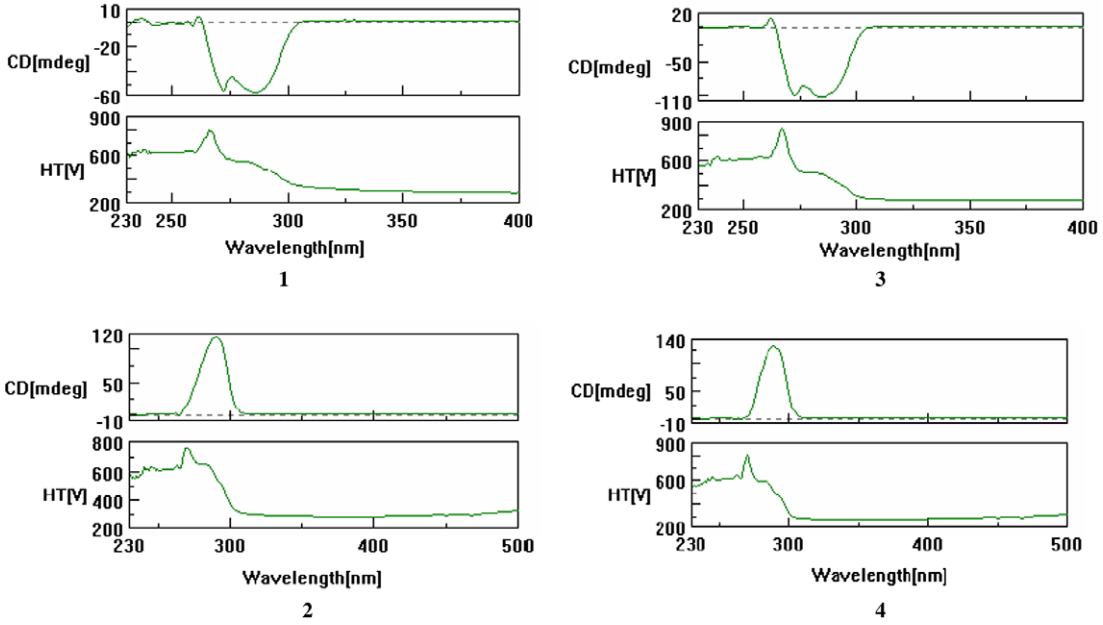

Fig. 2. CD spectra of compounds **1**–**4**.

imino and hydroxyl group. Bands due to an ester carbonyl, oxindole carbonyl and cyclic ether were present at ν_{max} 1721, 1705, and 1103 cm^{-1}, respectively. The absorption bands at 1622 and 750 cm^{-1} indicated the presence of benzene ring. However, though the ^1H and ^{13}C NMR spectra showed it to be isomeric with **1**, the chemical shifts observed in both the NMR spectra bear a closer resemblance to those of mitraphylline (**4**) for rings A, B, and C, rather than isomitraphylline (**3**). In isolate **2** also the absorption positions for the ring A, B, C, and D, were more or less identical to that of **4** with differences in the chemical shifts observed for the ring E in the ^1H and ^{13}C NMR. As a three proton doublet for C-18 methyl was observed at δ 1.24, showing connectivity with a carbon signal at δ 14.6 in the HSQC spectra, and not as a triplet, it could be inferred that the ring E is a closed one similar to **4**. The signal due to the olefinic bond in **4** was absent in **2** with appearance of two new absorption signals at δ 2.24 (1H, *m*) and 5.00 (1H, *d*, $J = 8.4$ Hz) showing connectivity with carbon signals at δ 56.2 and 91.5, respectively, in the HSQC experiment. That these two protons are adjacent to each other was visible from the coupling showed by them in the ^1H-H COSY experiment.

In the ^1H NMR, signals for four protons were observed in the aromatic region at δ 6.88 (1H, *d*, $J = 7.5$ Hz, H-12), 7.04 (1H, *ddd*, $J = 7.5$, 7.5, 0.8 Hz, H-10), 7.18 (1H, *ddd*, $J = 7.5$, 7.5, 0.8 Hz, H-11), and 7.20 (1H, *d*, $J = 7.5$ Hz, H-9), showing correlation with carbon signals at δ 110.0, 122.9, 128.4, and 123.3, respectively, indicating that similar to **4** the aromatic ring, i.e., ring A, is unsubstituted. The presence of two pairs of non-equivalent gem methylene protons at δ 1.23 (1H, *m*, H-14β), 2.39 (1H, *ddd*, $J = 11.0$, 2.8, 2.4 Hz, H-14α), and δ 1.70 (1H, *dd*, $J = 10.8$, 10.8, H-21α), 3.22 (1H, *dd*, $J = 10.8$, 3.0, H-21β) was revealed by the HSQC experiment. The former pair showed coupling with a proton signal at δ 2.42 (1H, *dd*, $J = 11.0$, 2.4 Hz). This proton signal also showed HMBC connectivity's with three other methylene group protons as well. Therefore, this signal at δ 2.42, which correlated with a carbon signal at δ 74.3 in the HSQC experiment, had to be assigned as H-3 since this was the only position at which a proton could have connectivity with four methylene groups. Typical axial–axial coupling constant ($J = 10.8$ Hz), was observed between C-21 proton at δ 1.70 and C-20 proton at δ 2.11. Therefore, this C-21 proton was assigned α-orientation. A signal at δ 2.48 (*m*) integrated for two protons. HSQC experiment showed it to be correlated with two different carbon signals at δ 35.1 and 54.5 as well as with two other proton signals at δ 3.36 (1H, *m*) and 2.04 (1H, *m*). The signals at δ 2.04, and 2.48 were assigned to 6α-H and 6β-H, respectively, as connectivities with protons of C-3, C-5, and the oxindole carbonyl signal at δ 181.7 were observed in the HMBC spectra. The remaining proton signals at δ 2.48, and 3.36, with carbon connectivity at δ 54.5 were assigned to 5α-H and 5β-H, respectively, on the basis of HMBC experiment in which connectivity with the protons of C-6, C-3, and C-21, was observed. Comparison of the signals for ring E of **2** in both ^1H and ^{13}C NMR spectra showed that similar to **1** there was a resemblance to **5** rather than **4** or **3**. The ester methyl appeared as a singlet at δ 3.59. The hydrogenation of the olefinic bond at 16, 17 position and introduction of a hydroxyl group was evident from the increase of 18 mass units in FABMS of **2** when compared with that of **4** and IR spectrum which showed additional absorption band due to hydroxyl group. Introduction of hydroxyl group in ring E can take place only at position C-17 as at C-16 there is an substitution of ester group and C-19 has a methyl group. The proton of C-19 appeared at δ 4.18 (1H, *qd*, $J = 6.2$, 3.0 Hz) as it showed coupling to C-18 and C-20 protons in the COSY experiment. The H-16 appeared as a multiplet at δ 2.24 while H-17 resonated at δ 5.00 as a doublet. As the chemical shifts in the ^1H and ^{13}C NMR correlated with the 17β-anomer of ajmalicinine rather than that for 17α-anomer, the hydroxyl group at C-17 was assigned β-orientation.

HMBC experiment established the assignments of quaternary carbons of **2** in a similar manner as in **1**.

The stereochemistry at various stereogenic centres was assigned in a similar manner as for **1**, **3**–**4** and it was found that **2** is also a normal type heteroyohimbine oxindole alkaloid. The C/D ring junction was found to be *trans* as is the case for normal type oxindole alkaloids on the basis of CD spectra (Fig. 2) which was comparable to mitraphylline (**4**). However, it differed from **1** in having 7(*R*) configuration as deduced from the fact that chemical shifts observed for protons and carbons at the C-3 and C-9 positions, were at δ 2.42 and 7.20, typical for a 7(*R*) isomer. The stereochemistry at other stereogenic centres was similar to **1**.

Acetylation of **2** resulted in the formation of a monoacetate **2a**. The C-17 methine was shifted downfield by about δ 1.46.

Thus, the structure of isolate **2** was deduced as 16, 17-dihydro-17β-hydroxy mitraphylline with the stereochemistry as 3*S*, 4*R*, 7*R*, 15*S*, 19*S*, 20*R*.

3. Experimental

3.1. General

Melting points (uncorrected) were determined on Toshniwal apparatus. NMR spectra were recorded in $CDCl_3$ for ^1H NMR at 300 MHz and for ^{13}C NMR at 75 MHz on a Bruker Avance 300 MHz spectrometer. Mass spectra were run on a JEOL SX 102 mass spectrometer. The IR spectra were taken on a Perkin–Elmer FT-IR spectrometer. R_f of compounds **1**–**4** were measured on Si gel $60F_{254}$ (Merck) readymade aluminium plates using $CHCl_3$:CH_3COCH_3 (5:4) as the mobile phase. The alkaloids were detected with an UV lamp, Dragendroff, and $FeCl_3/HClO_4$ spray reagents. Si gel (60–120 mesh) was used for purification of alkaloids in column chromatography.

3.2. Plant material

M. parvifolia leaves were collected in February 2005 from Lucknow and a voucher specimen (No. CIMAP-9035) has been deposited in the herbarium of this institute.

3.3. Extraction and isolation

Air dried and finely powdered leaves of *M. parvifolia* (3.5 kg) were extracted with ethanol at room temperature during 72 h. The ethanolic extract was concentrated under reduced pressure to yield a viscous mass (572 g). This extract was defatted with *n*-hexane and then treated with 3% HCl. This acidified extract was further fractionated with ethyl acetate and the remainder acidified aqueous extract was basified with ammonia in ice-cold conditions to pH 9. The basified extract was taken up in $CHCl_3$, washed with water, dried over anhy. Na_2SO_4 and concentrated under reduced pressure. The $CHCl_3$ fraction (2.5 g) was then subjected to column chromatography on Si gel (260 g) (60–120 mesh, Thomas Baker), with hexane/$CHCl_3$/MeOH mixture of increasing polarity. A total of 635 subfractions (ca. 100 ml each) were collected and combined on the basis of TLC analysis. Fractions eluted with a mixture of hexane–$CHCl_3$ (25:75) yielded **3** (136 mg) while those with a ratio of 10:90 afforded **4** (630 mg). **1** (128 mg) and **2** (106 mg) were obtained from fractions eluted with a mixture of $CHCl_3$:MeOH in the ratio 98:02.

3.4. 16,17-Dihydro-17β-hydroxy isomitraphylline (*1*)

Viscous mass; $[\alpha]_D + 0.052°$ ($CHCl_3$); UV λ_{max}: 211, 256, 286, 322 nm; IR υ_{max} cm^{-1}: 3423, 3250, 1720, 1705, 1622, 1102, 753; ^1H NMR (300 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (75 MHz, $CDCl_3$): see Table 2; FABMS *m/z* (rel. int.): 387 $[M + H]^+$ ($C_{21}H_{26}N_2O_5$) (66), 369 [387-$H_2O]^+$(40).

3.4.1. Acetylation of *1*

Compound **1** (0.005 g) was dissolved in pyridine (1 ml), and Ac_2O (1 ml) was added. After being left overnight at room temp. it was diluted with cold H_2O (25 ml) and then extracted with Et_2O (4 × 25 ml). The Et_2O extract was washed successively with dil. HCl (2 × 25 ml), $NaHCO_3$ soln. (2 × 25ml) and H_2O (2 × 25ml), and dried over Na_2SO_4. ^1H NMR (300 MHz, $CDCl_3$): δ 6.46 (1H, *d*, $J = 8.5$ Hz, H-17).

3.5. 16,17-Dihydro-17β-hydroxy mitraphylline (*2*)

Viscous mass; $[\alpha]_D - 0.053°$ ($CHCl_3$); UV λ_{max}: 209,256, 286, 321 nm; IR υ_{max} cm^{-1}: 3371, 3254, 1721, 1705, 1622, 1600, 1103, 750; ^1H NMR (300 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (75 MHz, $CDCl_3$): see Table 2; FABMS *m/z* (rel. int.): 387 $[M + H]^+$ ($C_{21}H_{26}N_2O_5$) (100), 369 [387-$H_2O]^+$ (18).

Table 1
^1H NMR shifts in δ for compounds **1**–**2** (in $CDCl_3$)

H	1	2
3	2.52, *dd*, $J = 11.5$, 3.2 Hz	2.42, *dd*, $J = 11.0$, 2.4 Hz
5α	2.54, *ddd*, $J = 8.9$,8.9,8.9 Hz	2.48, *m*
5β	3.30, *ddd*, $J = 8.9$, 8.0, 2.1 Hz	3.36, *m*
6α	2.04, *ddd*, $J = 13.0$, 8.9, 8.6 Hz	2.04, *m*
6β	2.41, *ddd*, $J = 13.0$, 8.9, 2.3 Hz	2.48, *m*
9	7.31, *d*, $J = 7.6$ Hz	7.20, *d*, $J = 7.5$ Hz
10	6.97, *ddd*, $J = 7.6$, 7.6, 0.8 Hz	7.04, *ddd*, $J = 7.5$, 7.5, 0.8 Hz
11	7.14, *ddd*, $J = 7.6$, 7.6, 1.2 Hz	7.18, *ddd*, $J = 7.5$, 7.5, 0.8 Hz
12	6.87, *d*, $J = 7.6$ Hz	6.88, *d*, $J = 7.5$ Hz
14α	2.21, *ddd*, $J = 11.5$, 3.0, 3.0 Hz	2.39, *ddd*, $J = 11.0$, 2.8, 2.4 Hz
14β	0.69, *ddd*, $J = 11.5$, 11.5, 11.5 Hz	1.23, *m*
15	1.95, *m*	1.95, *m*
16	2.24, *m*	2.24, *m*
17	4.99, *d*, $J = 8.4$ Hz	5.00, *d*, $J = 8.4$ Hz
18	1.17, *d*, $J = 6.8$ Hz	1.24, *d*, $J = 6.2$ Hz
19	4.14, *qd*, $J = 6.8$, 3.7 Hz	4.18, *qd*, $J = 6.2$, 3.0 Hz
20	1.85, *m*	2.11, *m*
21α	1.92, *dd*, $J = 11.0$, 11.0 Hz	1.70, *dd*, $J = 10.8$, 10.8 Hz
21β	3.11, *dd*, $J = 11.0$, 7.3 Hz	3.22, *dd*, $J = 10.8$, 3.0 Hz
23	3.55, *s*	3.59, *s*
NH	8.14, *brs*	8.85, *s*

Table 2
^{13}C NMR shifts in δ for compounds **1**–**2** (in $CDCl_3$)

Carbon No.	1	2
2	181.7	181.6
3	71.2	74.3
5	53.9	54.5
6	35.7	35.1
7	56.8	56.6
8	133.7	133.4
9	125.3	123.3
10	122.9	122.9
11	128.1	128.4
12	109.9	110.0
13	140.5	141.3
14	30.6	29.9
15	34.7	34.8
16	56.6	56.2
17	91.2	91.5
18	14.7	14.6
19	72.2	72.1
20	41.4	41.1
21	54.5	54.8
22	172.6	172.9
23	52.1	52.0

3.5.1. Acetylation of *2*

Compound **2** (0.005 g) was acetylated in a similar manner as **1**. ^1H NMR (300 MHz, $CDCl_3$): δ 6.46 (1H, *d*, $J = 8.5$ Hz, H-17).

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