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New class of steroidal alkaloids from *Fritillaria imperialis*

M. Nadeem Akhtar[a], Atta-ur-Rahman[a], M. Iqbal Choudhary[a,*], Bilge Sener[b], Ilkay Erdogan[b], Yoshisuke Tsuda[a]

[a]*International Center for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan*
[b]*Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara-06330, Turkey*

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Abstract

Two members of a new class of *C*-nor-D-homo steroidal alkaloids, impranine (**1**) and dihydroimpranine (**2**) along with a new pyridyl-pregnane-type steroidal alkaloid, fetisinine (**3**) and a known base, korsevine (**4**) were isolated from the bulbs of *Fritillaria imperialis*. The structures of the compounds were established on the basis of spectroscopic techniques and some chemical transformations. Compounds **1** and **2** form a new class of steroidal alkaloids, named as ''impranane.''

Keywords: *Fritillaria imperialis*; Impranine; Dihydroimpranine; Fetisinine; Impranane-type steroidal alkaloids

1. Introduction

The genus *Fritillaria* (Liliaceae) comprises about 100 species and has a distribution in the temperate regions of the northern hemisphere (Tsukamoto, 1989). A few species are native to Cyprus, southern Turkey and Iran (Ori et al., 1992). In China, the bulbs of *Fritillaria* species have been used as antitussive and expectorant agents (Kaneko et al., 1988). The crude drug comprising ''Fritillarae bulbs'' (''Bi-mu or Pei-mu'' in Chinese and ''Bai-mo'' in Japanese) has been long used as a principal Chinese medicine (Kitajima et al., 1981). It is also reported to possess blood platelet aggregation inhibitory activity (Sook et al., 1986). *Fritillaria imperialis* has been used for the treatment of various ailments such as sore throat, cough, asthma, bronchitis, scrofula, gland tumor, dysuria and haemoptysis in folklore (Bailey, 1966; Perry, 1980). Our previous studies on this plant have resulted in the isolation of a number of cholinesterase inhibiting (Atta-ur-Rahman et al., 2002) and anticholinergic (Atta-ur-Rahman et al., 1994, 1998) steroidal alkaloids. The present paper describes the isolation of three new steroidal bases, impranine (**1**), dihydroimpranine (**2**), and fetisinine (**3**) along with a known alkaloid, korsevine (**4**).

2. Results and discussion

The alkaloidal fraction of the bulbs of the plant were extracted with chloroform at pH 9–11 and subjected to column chromatography on silica gel using hexane-acetone mixtures as eluent. After repeated column and thin layer chromatography, two novel *C*-nor-D-homo steroidal alkaloids **1** and **2**, and a new pregnane-type steroidal alkaloid **3** together with a known base korsevine (**4**) were isolated. The structures of the compounds were established on the basis of 1D and 2D NMR spectroscopy.

Impranine (**1**) was isolated as an amorphous powder. The HR-EI-MS displayed the $[M]^+$ at *m*/*z* 427.3318 ($C_{28}H_{45}NO_2$, calc. 427.3316). The IR spectrum exhibited absorption bands for OH (3468–3456 cm^{-1}), carbonyl (1706 cm^{-1}), C=C double bond (1638–1634 cm^{-1}) and *N*-methyl (2785, 1455 cm^{-1}) groups. The position of the carbonyl group at C-6 was inferred from the CD spectrum, which showed a negative maximum at 292 nm, characteristic of 6-keto steroids.

The mass fragmentation pattern of compound **1** is similar to veratranine (Li et al., 1988) and veratramine-types steroidal alkaloids (Budzikiewicz, 1964). The base peak at *m*/*z* 112 could arise by the cleavage of

* Corresponding author. Tel.: +92-1-924-3211; fax: +92-1-924-2190.
E-mail address: zainraa@digicom.net.pk (M.I. Choudhary).

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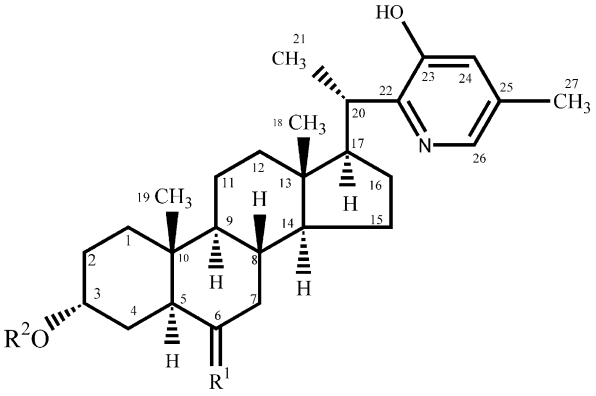
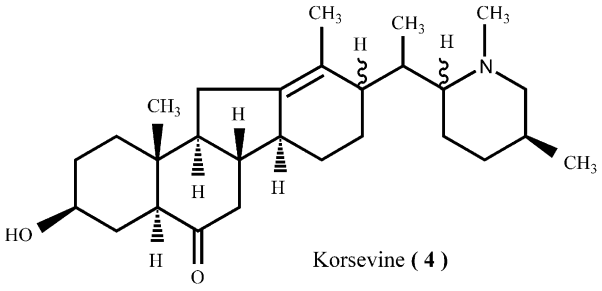
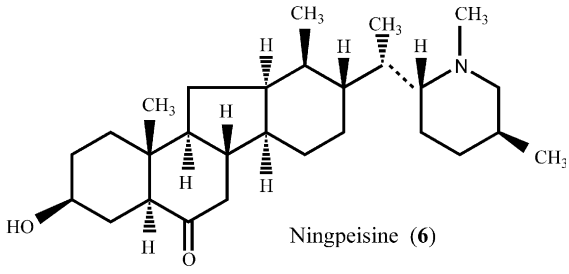
the C-20 and C-22 bond. Second major fragment at *m/z* 98 was attributed to the *N*-methyl piperidinium ion resulting from the loss of a C-25 methyl group from the ion at *m/z* 112. The fragment ion at *m/z* 139 could be formed by the simultaneous cleavage of C-17 and C-20 bond. Another important fragment at *m/z* 179 could be formed by RDA cleavage of ring D, and radical generated at C-13 was stabilized by the migration of a hydrogen atom from C-8 leading to another radical at C-8, which possibly stabilized by migration of a bond from C-9 to C-8. The overall fragmentation of **1** is similar to that of the known alkaloid ningpeisine (**6**), isolated from *F. ningguoensis* (Li et al., 1988) (Scheme 1).



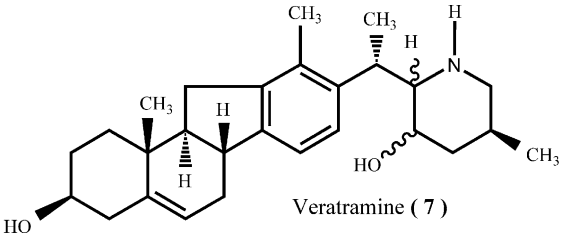
Fetisinine (**3**)	R^1 = O	R^2 = H
Dihydrofetisinine (**3a**)	R^1 = H (OH)	R^2 = H
Monoacetylfetisinine (**3b**)	R^1 = O	R^2 = Ac
Diacetylfetisinine (**3c**)	R^1 = Ac	R^2 = Ac



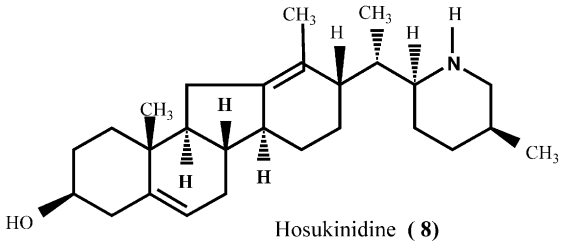
Korsevine (**4**)



Ningpeisine (**6**)

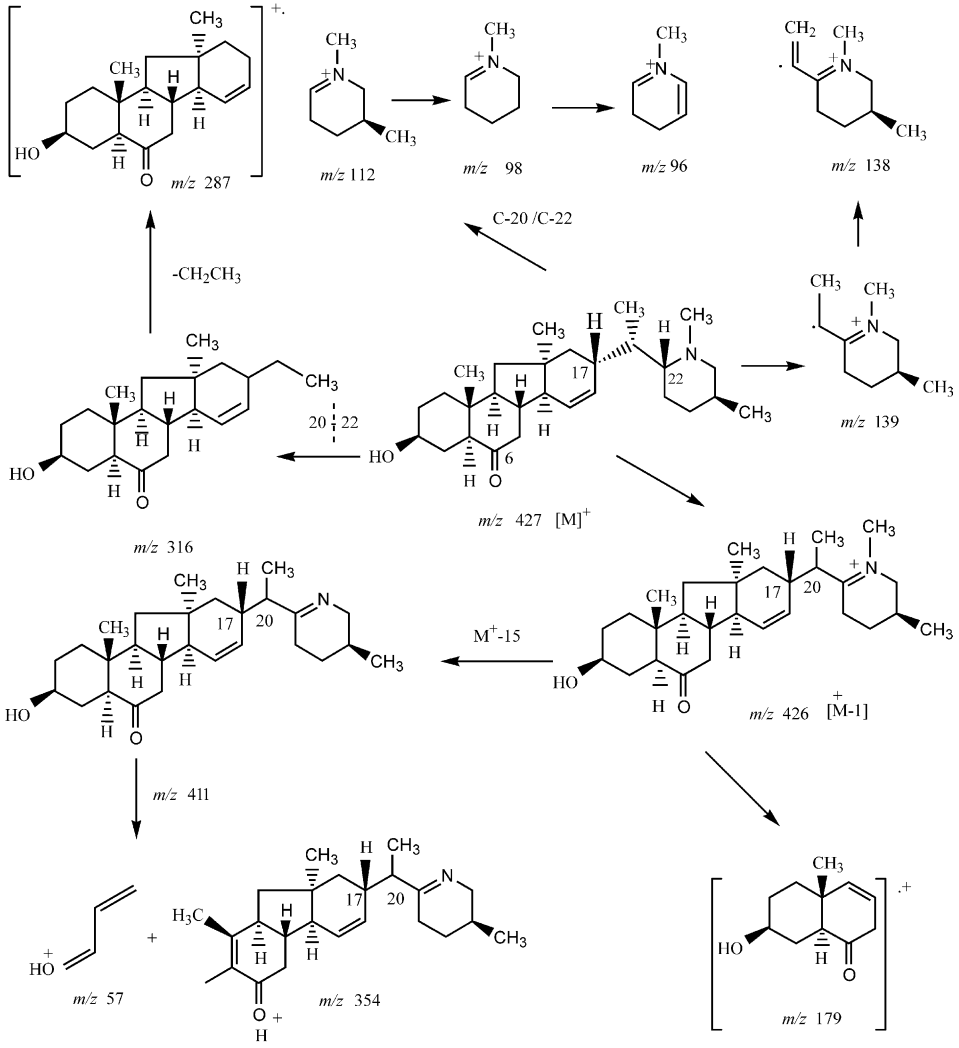


Veratramine (**7**)

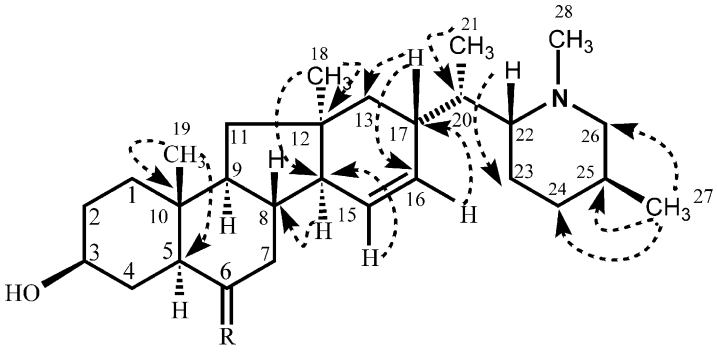


Hosukinidine (**8**)

The ^{1}H NMR spectrum of **1** exhibited five methyl signals, which include two tertiary methyl signals resonating at δ 0.79 (3H, *s*, H_3-19) and 1.20 (3H, *s*, H-18), two secondary methyls at δ 0.55 (*d*, $J_{20,21}$ = 6.2 Hz, H_3-21) and 0.88 (*d*, $J_{25,27}$ = 7.2 Hz, H_3-27), and one *N*-Me group at δ 2.25 (3H, *s*, H_3-28). The upfield chemical shift of the C-18 methyl signal in **1** indicated the unusual nature of the molecule, since the protons of C-18 methyl normally resonates between δ 1.6-2.3 in *C*-nor-D-homo steroidal alkaloids as in jervine and veratramine (**7**) (Shakirov and Younsov, 1980). Two signals resonating at δ 5.29 (*ddd*, $J_{15,16}$ = 15.2 Hz, $J_{15,14}$ = 8.2 Hz, $J_{15,17}$ = 2.9 Hz) and 5.45 (*ddd*, $J_{16,15}$ = 15.2 Hz, $J_{16,17}$ = 8.3 Hz, $J_{16,14}$ = 2.9 Hz) were assigned to the C-15 and C-16 protons of a disubstituted double bond, respectively. The ^{1}H–^{1}H COSY-45° spectrum showed cross-peaks between the olefinic H-15 (δ 5.29), and H-16 (δ 5.45), and between H-14 (δ 1.90) and H-17 (δ 2.15). The chemical shift of H-17 (δ 2.16) was also correlated with the two methylene protons, H_2-13 (δ 1.95 and 1.25). The HMBC spectrum of **1** showed distinct long-range correlations of H-15 (δ 5.29) with C-14 (δ 53.5), while H-16 (δ 5.45) showed correlation with C-17 (δ 43.4). On the other hand, H-14 (δ 1.90) showed correlation with C-15 (δ 132.0), while H-17 (δ 2.15) was correlated with C-15. The protons of H-18 (δ 1.20) showed correlations with C-12 (δ 29.5) and C-14 (δ 53.5). Other important HMBC correlations are shown in Fig. 1. These correlations have supported the position of the double bond between C-15 and C-16 and the presence of a methyl at C-12. Moreover, the ^{13}C NMR chemical



Scheme 1. Mass fragmentation of impranine (**1**).



Impranine (**1**) R = O

Dihydroimpranine (**2**) R = H (OH)

Fig. 1. ^{1}H–^{13}C long-range correlations of **1** observed in HMBC spectrum.

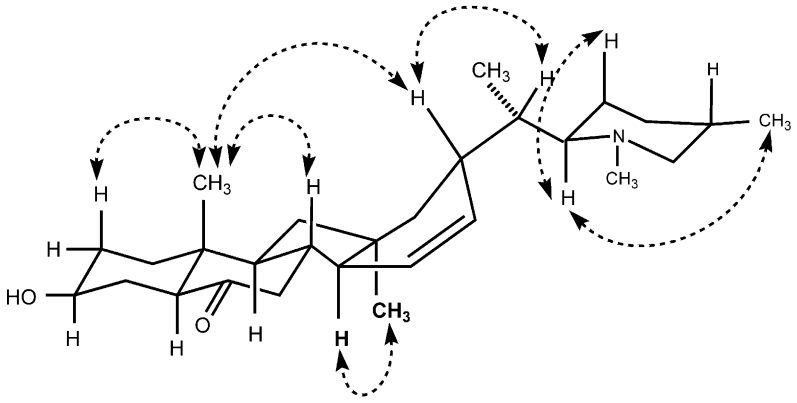


Fig. 2. Diagnostic NOEs and perspective view of impranine (**1**).

shifts of C-22 to C-28 in compound **1** are similar to those reported for ningpeisine (**6**) (Li et al., 1988), further supporting the presence of an *N*-methyl piperidy moiety in impranine (**1**). A multiplet at δ 3.52 was assigned to the H-3α geminal to the hydroxyl group. The half-width of the C-3 proton signal ($W_{1/2}$ = 24.0 Hz) indicated its axial orientation. The relative configuration at C-3 was further confirmed by the Horeau's method (Horeau, 1961; Fiaud et al., 1977) through esterification of compound **1** with 2-phenylbutyric anhydride. The resulting 2-phenyl butanoic acid was found to be dextrorotary (*R*), suggesting the (*S*) configuration at C-3 (Eliel et al., 1994). The stereochemistry at other chiral centers were deduced on the basis of chemical shift comparison and NOESY interactions as summarized in Fig. 2. H-19 (δ 0.79) showed NOESY cross-peaks with H-17 (δ 2.16), H-2 (δ 1.87) and H-8 (δ 1.68). Similarly, H-22 (δ 2.20) also showed cross-peaks with H-23 (δ 1.56), H-20 (δ 1.93) and H-27 (0.88), but not with H-18. This indicated that the ring C has an envelop form and with *cis* ring junction.

The relative configurations at C-17 and C-22 were inferred as *R* by spectral comparison with the reported compounds, ningpeisine (**6**) and hosukinidine (**8**) (Kaneko et al., 1970a, 1979) and on biogenetic considerations since the substance probably arises via *C*-nor-D-homo rearrangement of epirubijervine and successive cleavage of C-16-*N* bond (Kaneko et al., 1970b). The stereochemistry at C-20 was assigned to be *S*, consistent with the *S* configuration of C-20 (α-methyl) in all naturally occurring steroidal alkaloids (Kaneko et al., 1979). On the basis of these spectral evidences, the structure of impranine (**1**) was deduced as (3*S*, 17*R*, 20*S*, 22*R*,)-5α-impra-15,16-ene-6-one, representing the first member of a new class of steroidal alkaloids.

The biosynthesis of the *Veratrum* skeleton is well-studied (Kaneko et al., 1970a,b, 1979). We propose here the possible biogenetic route to this new "impranane" class arises from the veratrainine skeleton. Protonation of the known (Jerveratrum) intermediate (A) can yield carbocations (B) and (C). Subsequent 1,2-methyl shift from C-12 to C-13 in carbocation species (C) could lead to a new cation (D). The deprotonation of cation (D) followed by a 1,2-hydride shift can yield this new class of *C*-nor-D-homo-steroidal alkaloid "impranane" (**I**). Although the suggested pathway in Scheme 2 includes some high energy intermediates such as (E), the C/D *cis* with α-orientation of the side chain is expected to be the most preferable among other possible stereoisomers. The ring junction and orientation of side chain is energetically more stable (*J*) as inferred from 3D-energy minimized calculation.

Dihydroimpranine (**2**) was isolated as an amorphous powder. The $[M]^+$ at *m*/*z* 429.4536 (calc. 429.4526 for $C_{28}H_{47}NO_2$) was found to be 2 a.m.u. higher than that of **1**. The characteristic fragments at *m*/*z* 98 and 112 (100%) indicated that the compound **2** also contained an *N*-methyl piperidyl side chain moiety. Its mass fragmentation pattern was also identical to that found in veratranine-type alkaloid, ninigpeisine (**6**) (Li et al., 1988). The IR spectrum showed strong absorptions for OH (3441–3438 cm^{-1}), $-CH_2$ (2906 cm^{-1}), *N*-methyl (2765, 14565 cm^{-1}) and C=C (1636 cm^{-1}) functionalities.

The ^1H NMR spectrum of **2** exhibited five methyl signals which include two tertiary methyls at δ 0.62 (3H, *s*, H_3-19) and 1.19 (3H, *s*, H_3-18), two secondary methyls at δ 0.88 (*d*, $J_{25,27}$ = 6.3 Hz, H_3-27) and 0.56 (*d*, $J_{20,21}$ = 6.4, H_3-21) and an *N*-CH_3 signal at δ 2.30. The hydroxyl group at C-3 was deduced to be equatorially oriented, since its geminal proton resonating δ 3.65 showed a $W_{1/2}$ = 23.0 Hz, corresponding to its axial orientation. Another downfield signal at δ 3.80 ($W_{1/2}$ = 12.3 Hz) was assigned to the H-6 geminal to an equatorially oriented hydroxyl group. Two olefinic protons resonated at δ 5.60 (ddd, $J_{15,16}$ = 15.0 Hz, $J_{15,14}$ = 8.5 Hz, $J_{15,17}$ = 2.9 Hz, H-15) and 5.45 (*ddd*, $J_{16,15}$ = 15.1 Hz, $J_{16,17}$ = 8.5 Hz, $J_{16,14}$ = 2.9 Hz, H-16).

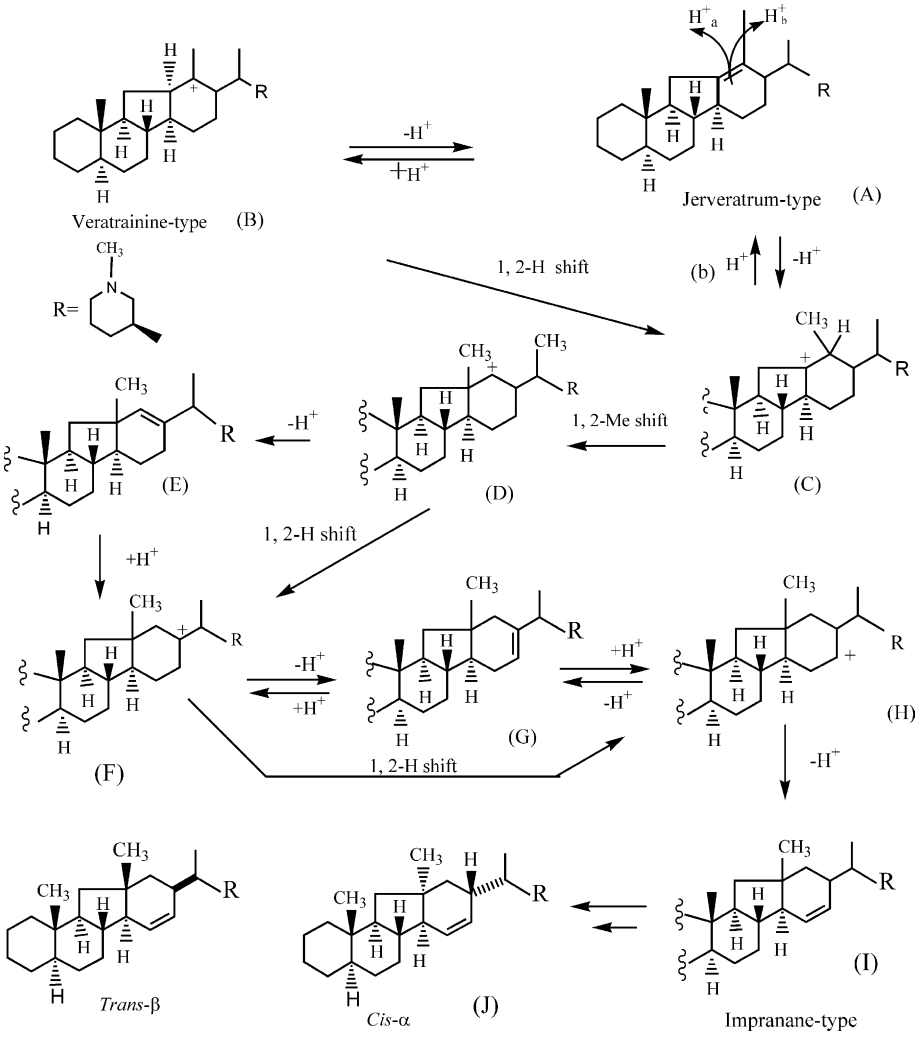
The position of the double bond was inferred from COSY 45°, HMQC and HMBC experiments.

Compound **2** appeared to be a C-6 reduced derivative of impranine (**1**). This was confirmed by the reduction of **1** with $NaBH_4$ to obtain dihydroimpranine. The reduced product was found to be spectroscopically and chromatographically identical to dihydroimpranine (**2**). The chemical shift of H-19 (δ 0.79) (0.14 ppm downfield from the normal value) was due to a 1, 3-diaxial interaction of the C-19 methyl indicating the presence of a hydroxyl group at C-6 (Auria et al., 1984). The structure of compound **2** was therefore deduced to be (17*R*, 22*S*, 25*S*)-5α-impra-15, 16-ene, 3β, 6β-diol.

New compound fetisinine (**3**) was obtained as a colorless amorphous powder. The molecular formula was deduced by HR-EI-MS $[M]^+$ to be $C_{27}H_{39}NO_3$ (*m*/*z* 425.2829, calc. 425.2836). The UV spectrum showed absorptions at 287, 220 and 202 nm consistent with the presence of a hydroxy pyridine moiety (Katritzky, 1963). The IR spectrum displayed strong absorptions for OH (3433–3387 cm^{-1}), pyridine moiety (1525–1515, 1587–789 cm^{-1}) and carbonyl (1704 cm^{-1}) functionalities. The presence of a carbonyl group was further confirmed by a signal at δ 211.5 and the CD spectrum showed negative maximum at 302 (Schaffner and Snatzke, 1965).

The ^1H NMR spectrum of **3** exhibited four methyl signals. Two tertiary methyls appeared as an overlapping singlet at δ 0.50 (H_3-18 and H_3-19), while a 3H singlet resonated at δ 2.10 (H_3-27). A secondary methyl doublet appeared at δ 1.01 ($J_{20,21} = 7.0$ Hz, H_3-21). Two aromatic protons resonated at δ 6.80 and 7.80 were assigned to H-24 and H-26 of the pyridine moiety. The chemical shift of C-19 methyl protons experience a downfield shift by δ 0.12 ppm, when **3** was reduced with $NaBH_4$ to yield **3a**. This indicated that the hydroxyl group in the resulting 3α-diol **3a** has a 1,3-diaxial interaction with H_3-19 (Auria et al., 1984).

The presence of a pyridyl-pregnane moiety in compound **3** was also inferred from its mass spectrum since the fragmentation pattern was similar with that of the



Scheme 2. Proposed biogenetic pathway of impranane alkaloids.

known pyridyl-pregnane containing alkaloid, petisidinine (Nakhatov et al., 1983). The peak at m/z 136 was due to the cleavage of the C-17 and C-20 bond, while the base peak at m/z 137 arose through a proton shift to that ion. The mass fragmentation pattern of compound **3** suggested that it belongs to the verazine group of alkaloids *e.g*, verdinine and petisidinine (Shakirov et al., 1995; Nakhatov et al., 1983). The hydroxyl group at C-3 was inferred to be axially orientated, since its geminal proton appeared characteristically at δ 3.33 with $W_{1/2} = 12.5$ Hz, indicating its equatorial orientation (Eliel et al., 1994; Kitajima et al., 1981). The chemical shift of the protons geminal to the acetoxy group in **3b** (obtained by acetylation of **3**) shifted downfield and resonated at δ 3.55, while a signal at δ 1.97 in **3b** could be assigned to the acetoxy proton substituted at C-3. On acetylation of dihydrofetisinine (**3a**), diacetoxyfetisinine (**3c**) was obtained and the protons geminal to the acetoxy group were found to be resonated downfield at δ 3.56 and 4.22, which showed the presence of two secondary hydroxyl groups in **3c**, whereas two 3H singlets at δ 1.97 and 2.10 were assigned to the acetoxy protons at C-3 and C-6, respectively. The axial orientation of the C-3 hydroxyl group in **3** was further confirmed through the Horeau's method. On the esterifiction of compound **1** with 2-phenyl butyric anhydride, the resulting 2-phenyl butanoic acid was found to be levorotatory (*S*). This suggested an (*R*) configuration at C-3 (Eliel et al., 1994).

For the determination of absolute configuration at C-20, compound **3** was first reduced with $NaBH_4$ to alcohol **3a** to eliminate the contribution of C-6 carbonyl group and the CD spectrum of the resulting alcohol was measured. It showed a positive CD spectrum at 330 nm. Since the sign of pyridyl-pregnane chromophore depends on the chirality at C-20, and 20*S* derivatives are known to give a positive cotton effect (Snatzke, 1967), it was therefore concluded that compound **3** was a 20*S*-derivative. By careful examination of the spectroscopic data including CD spectrum, the structure of fetisinine (**3**) was deduced to be (3*R*, 20*S*)-3-hydroxyl-20-(5′-hydroxy-3′-methyl pyridin-6′-yl)-5α-pregnan-6-one (**3**).

Compound **4** ($C_{28}H_{45}O_2N$), crystallized from the acetone, showed a UV absorption at 290 nm. The spectral data including ^1H NMR, IR and mass fragmentation pattern was identical with the korsevine (**4**) isolated earlier from the bulbs of *Korolkowia sewerzowii* (Nuriddinov and Younusov, 1967). However this is the first report of its isolation from the title plant.

Table 1
^{13}C NMR data of compounds (**1–3** and **6**)

Carbon no.	Compound no.			
	1	**2**	**3**	**6**
1	37.0	38.2	37.0	37.1
2	33.3	33.1	34.2	33.8
3	70.1	70.2	69.7	70.8
4	31.4	30.9	29.2	31.6
5	56.[4a]	56.3	56.6	56.4
6	211.5	69.8	211.0	210.6
7	46.4	46.0	46.2	46.7
8	39.9	38.6	37.3	41.8
9	56.5[a]	55.0	53.5	56.9
10	35.5	35.8	36.0	37.7
11	30.5	29.8	28.7	30.9
12	37.6	37.3	36.3	40.8
13	29.5	30.1	40.6	38.1
14	53.5	54.0	38.0	43.0
15	132.0	133.1	27.2	24.2
16	134.1	134.5	21.4	21.7
17	43.4	42.3	55.5	48.1
18	13.1[a]	12.8	12.9	12.6
19	13.0[a]	12.7	12.5	12.9
20	37.5	36.7	46.3	31.8
21	13.2	14.2	17.7	12.0
22	67.0	68.2	177.0	66.0
23	21.0	22.1	140.2	21.6
24	29.6	30.6	123.0	30.9
25	31.0	31.6	131	31.6
26	53.0	53.2	123.0	53.8
27	19.0	19.6	16.6	19.8
N–CH_3	42.8	43.0	–	43.4

[a] Assignments are interchangeable.

3. Experimental

3.1. General

Melting points: Yanaco Micro Melting Point apparatus. Optical rotation: Jasco DIP-360 digital polarimeter with a 10-cm cell. UV spectra: Hitachi UV-3200 spectrophotometer: λ_{max} (log ε) in nm. The IR spectra: Jasco A-302 spectrophotometer in $CHCl_3$ or KBr and ν in cm^{-1} CD spectra: Jasco-*J*-600 spectropolarimeter nm ($\Delta\varepsilon$). ^1H and ^{13}C NMR, COSY, HMBC, and HMQC spectra: ($CDCl_3$ or $CDCl_3/CD_3OD$) Bruker spectrometers with 400, 100 and 500 MHz; chemical shifts δ in ppm and coupling constants J in Hz, relative to $SiMe_4$ (TMS) as internal standard. Mass spectra (MS) and HR-EI-MS: Varian MAT 312 double focussing spectrometer: m/z (rel. int. %). CC silica gel = 70–270 mesh (ASTM Merck). Flash chromatography: silica gel, 230–400 mesh (ASTM, Merck). TLC Merck silica gel precoated TLC cards. Alkaloids were detected by Dragendroff's reagent.

3.2. Plant material

The bulbs of *F. imperialis* (K. Fragner) (40 kg) were harvested at the flowering stage from Alanya, Turkey, October 1997 and a voucher specimen (# 59361) was deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey.

3.3. Extraction and isolation

The air-dried bulbs of *F. imperialis* were powdered and percolated in EtOH (25 l) at room temperature (2 weeks) to afford crude extract (350 g). This was suspended in water (3 l), extracted successively with hexane, chloroform (pH, 3–3.5), chloroform (pH 9–11), ethyl acetate and butanol, concentrated to afford, 72.2, 75.30, 11.3, 15.35 and 20.4 g extracts, respectively. The chloroform extract at pH 9–11 (11.3 g) was loaded on a silica gel column and eluted with a gradient solvent system of chloroform/petroleum ether mixture and 58 sub-fractions were obtained. The fractions 14–47 were combined (6.5 g) and subjected to silica gel column chromatography. After repeated column chromatography using acetone–pet.ether and a few drops of diethylamine, impranine (**1**) (18.6 mg, $R_f = 0.64$), dihydroimpranine (**2**) (23.3 mg, $R_f = 0.50$), fetisinine (**3**) (35.0 mg. $R_f = 0.46$), and korsevine (**4**) (16.23 mg, $R_f = 0.57$), were purified.

3.4. Impranine (3S, 17R, 20S, 22R)-5α-impra-15, 16-ene-6-one (1)

Amorphous powder (18.6 mg), R_f 0.64 (25% acetone/hexane, drops of diethylamine), $[\alpha]_D^{25} = +28$ ($c = 0.05$, MeOH). UV λ_{max}^{MeOH}(log ε): 289 (2.47). CD: MeOH ($\Delta\varepsilon$); 292 nm (−1.95). IR ν_{max} (KBr): 3468–3456, 2785, 1706, 1638–1634, 1485. ^{1}H NMR ($CDCl_3/CD_3OD$, 400 MHz) δ: 3.52 (*br*, *m*, $W_{1/2} = 24.0$ Hz, H-3α), 1.88 (*m*, H-5α)?, 1.90 (*t*, H-14), 5.29 (1H, *ddd*, $J_{15,16} = 15.2$ Hz, $J_{15,14} = 8.2$ Hz, $J_{15,17} = 2.9$ Hz, H-15), and 5.45 (1H, *ddd*, $J_{16,15} = 15.2$ Hz, $J_{16,17} = 8.3$ Hz, $J_{16,14} = 2.9$ Hz, H-16), 2.15 (*m*, H-17), 1.20 (3H, *s*, H-18), 0.79 (3H, *s*; H-19); 0.55 (3H, *d*, $J_{20,21} = 6.4$ Hz, H-21), 2.20 (m, H-22), 1.30 (m, H-24), 2.80 (*dd*, H-26), 0.88 (*d*, $J_{25,27} = 7.2$ Hz, H-27), 2.25 (s, H-C(28). EI-MS *m*/*z* (rel. int.%); 427 (86), 112 (100), 98 (80), 412 (33), 356 (10), 314 (6), 218 (4), 287 (4). HR-EI-MS *m*/*z* 427.3318 (calc. 427.3316 for $C_{28}H_{45}NO_2$). ^{13}C NMR: δ Table 1.

3.5. Dihydroimpranine (17R, 20S, 22R)-5α-impra-15, 16-ene-3β, 6β-diol (2)

Amorphous powder (23.3 mg), R_f 0.50 (30% acetone/hexane, drop of diethylamine) $[\alpha]_D^{25} = -32$ ($c = 0.08$ MeOH). IR υ_{max}^{KBr} cm^{-1}: 3441–3428, 2906, 2765, 1636, 1485. ^{1}H NMR ($CDCl_3/CD_3OD$, 400 MHz) δ 3.65 (*br*, *m*, $W_{1/2} = 23.0$ Hz, H-3α), 1.94 (m, H-5α), 3.80 (1H, *br*, *m*, $W_{1/2} = 12.0$ Hz, H-6α), 5.60 (1H, *ddd*, $J_{15,16} = 15.0$ Hz, $J_{15,14} = 8.5$ Hz, $J_{15,17} = 2.8$ Hz, H-15), 5.45 (1H, *ddd*, $J_{16,15} = 15.1$ Hz, $J_{16,17} = 8.3$ Hz, $J_{16,14} = 2.9$ Hz, H-16), 2.17 (1H, *m*, H-17), 1.19 (3H, *s*; H-18),. 0.65 (3H, *s*; H-19), 0.56 (3H, *d*, $J_{20,21} = 6.4$ Hz, H-21), 2.23 (1H, *m*, H-22), 0.88 (3H, *d*, $J_{25,27} = 7.0$ Hz, H-27), 2.30 (3H, *br*, *s*, H-28), EI-MS: (rel. int.%); *m*/*z* 429 (48), 416 (51), 415 (91), 414 (83), 400 (34), 386 (26), 356 (49), 112 (100), 98 (67). HR-EI-MS: *m*/*z* 429.4536 (calc. 429. 4526 for $C_{28}H_{47}O_2N$). ^{13}C NMR: δ Table 1.

3.6. Fetisinine (3R, 20S)-3-hydroxyl-20-(5′-hydroxy-3′-methylpyridin-6′-yl)-5α-pregnan-6-one) (3)

Amorphous colorless powder, (35.0 mg) R_f 0.46, $[\alpha]_D^{25} = -118$ ($c = 0.10$, MeOH). UV λ_{max}^{MeOH} nm (log ε: 287 (3.48), 220 (3.38), 202 (3.85). CD spectrum MeOH ($\Delta\varepsilon$) ($c = 0.2$, MeOH): 302 (−1.09), 328 (0.08). IR ($CHCl_3$) cm^{-1}: 3433, 2904, 1704, 1525–1515 1585, 789. ^{1}H NMR ($CD_3OD/CDCl_3$, 400 MHz): δ 0.50 (6H, *s*, H-18 and H-19), 1.01 (3H, *d*, $J_{20,21} = 7.0$ Hz, H-C-21). 2.10 (3H, *s*, H-27), 1.55 (H-17), 3.33 (1H, *br*. *m*, $W_{1/2} = 12.5$ Hz, H-3β), 6.80 and 7.80 (1H, *s*, each 1H, H-24 and H-26), HR-EI-MS: *m*/*z* 425.2829 (calc. 425.2836 for $C_{27}H_{39}NO_3$). EI-MS: (rel. int.,%) *m*/*z* 425 (88), 410 (90), 356 (11) 285 (5), 244 (24), 190 6), 177 (55), 176 (60), 164 (25), 137 (100), 136 (89), (162), (70), 123 (80), 110 (15), 93 (25). ^{13}C NMR: δ Table 1.

3.7. Dihydrofetisinine (3α, 6β-dihydroxy-20S-(−5′-hydroxy-3′-methylpyridin-6′-yl)-5α-pregnan) (3a)

Fetisinine (**3**) (10 mg) in MeOH (10 ml) was treated with $NaBH_4$ (46 mg) and the solution was left for at room temperature 6 h. The reaction mixture was poured into water and extracted with chloroform and dried over Na_2SO_4 to yield dihydrofetisinine (**3a**) (8.5 mg) R_f 0.47. I.R. (KBr) cm^{-1}: 3502–3497, 1536, 1534, 1587, 764; UV MeOH (log ε): 287 (−2.47), CD: MeOH ($\Delta\varepsilon$), +330 (0.20), ^{1}H NMR ($CDCl_3/CD_3OD$, 300 MHz), δ 0.61 (3H, *s*, H-19), 0.63 (3H, *s*, H-18), 1.07 (3H, *d*, $J_{20,21} = 7.0$ Hz, H-21), 3.34 (H-3α) 6.80 (1H, *br*, *s*, H-24), 7.80 (1H, *s*, H-26), 2.12 (3H, *s*, H-27), EI-MS: *m*/*z* 427.2345 ($C_{27}H_{41}NO_3$).

3.8. Monoacetylfetisinine (3α-Acetoxy-20S-(5′-hydroxy-3′-methylpyridin-6′-yl)-5α-pregnan) (3b)

Fetisinine (**3**) (10 mg) was treated with a mixture of Ac_2O/pyridine (1/1.5 ml) at room temperature for overnight. The product was poured into distilled water and extracted with $CHCl_3$ concentrated and chromatographed over silica gel. This yielded the amophours powder of monoacetylfetisinine (**3b**) (8.40 mg) $R_f = 0.60$, ^{1}H NMR δ: 0.65 (3H, *s*, H-19), 0.67 (3H, H-18), 1.05 (3H, *d*, $J_{20,21} = 7.0$ Hz, H-21), 1.97 (3H, *s*, $OCOCH_3$), 2.21 (3H, *s*, H-27), 3.54 (1H, *br*, *m*,. H-3β), 6.80 (1H, *br*, *s*, H-24), 7.77 (1H, *br*, *s*, H-26); EI-MS: *m*/*z* 467. EI-MS HR $[M]^+$ *m*/*z* 467.4518 (calc; 467.4509 for $C_{29}H_{41}NO_4$).

3.9. Diacetyldihydrofetisinine (3α, 6β,-diacetoxy-20S-(5′-hydroxy-3′-methylpyridin-6′-yl)-5α-pregnan) (3c)

Compound **3b** (8.40 mg) was acetylated the same way as described in **3b** which yielded amorphous powder

(5.40 mg) with $R_f = 0.69$. ^{1}H NMR δ 0.66 (3H, *s*, H-19), 0.70 (3H, *s*, H-18), 3.57 (1H, *m*, H-3β), 4.45 (1H, *m*, H-6α), 1.05 (3H, *d*, $J_{20,21} = 6.9$ Hz, H-21), 1.97 (3H, *s*, $OCOCH_3$, at 3), 2.0 (3H, *s*, $OCOCH_3$, at C-6), 6.80 (1H, *br*, *s*, H-24), 7.79 (1H, *br*, *s*, H-26), HR-EI-MS *m*/*z* 511.2219 (calc. 511.2318 for $C_{31}H_{45}O_5N$) $[M]^+$ *m*/*z* 511.

3.10. Korsevine (4)

Needle from acetone, mp 170–172 °C, $[\alpha]_D^{25} = -87$ ($c = 0.5$, MeOH), UV λ_{max}^{MeOH} nm (log ε): 290 (1.93). I.R. $_{max}^{KBr}$ cm^{-1} 3252–3256, 2677, 1712, 1628, 2894, and 1485. ^{1}H NMR ($CDCl_3/CD_3OD$, 400 MHz), δ 0.60 (3H, *s*, H-19), 0.94 (3H, *d*, $J_{25,27} = 7.0$ Hz, H-27) 0.68‘(3H, *d*, $J_{20,21} = 6.5$ Hz, H-21), 1.56 (3H, *s*, H-18), 2.23 (3H, *s*, N-CH_3), 3.55 (1H, *m*, $W_{1/2} = 24.0$ Hz, H-3α), MS (rel. int.%), *m*/*z* 427 (80), 98 (100), 112 (12), 164 (10), 356 (8), FD *m*/*z* 427, HR-EI-MS: *m*/*z* 427.3312 (calc. 427.3458 for $C_{28}H_{45}NO_2$).

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