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IMPERIALIDIENE: NEW STEROIDAL ALKALOID PRODUCED BY MICROBIAL TRANSFORMATION OF IMPERIALINE, ISOLATION AND STRUCTURE ELUCIDATION.

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Abstract

Incubation of Imperialine (**1**) with the fungus *Cephalosporium aphidicola* yielded a new and a known compound. These compounds were fully Characterized and identified as $\Delta^{6,9(11)}$, 5a, 17b, 25S-cevanin, 3b-20a-diol, named Imperialidiene (**2**), and Imperialone (**3**) on the basis of modern spectroscopic techniques.

Keywords: Cephalosporium Aphidicola, Imperialine, Imperialidiene, and Imperialone.

INTRODUCTION

Steroidal alkaloids have been investigated for a wide range of potential bioactivities including anti-estrogenic, anti-inflammatory, antimicrobial and Chemotherapeutic[1], [2]. *Fritilaria imperialis* of Turkish origin and *Fritillaria unibracteata* which has found extensive use in traditional medicine are rich in steroidal alkaloids. Imperialine (**1**) is a major steroidal base of the plant, which exhibits moderate anticholinergic, inflammatory activity and anticancer activity[3]–[6]. The structure-activity relationship of compound **1**

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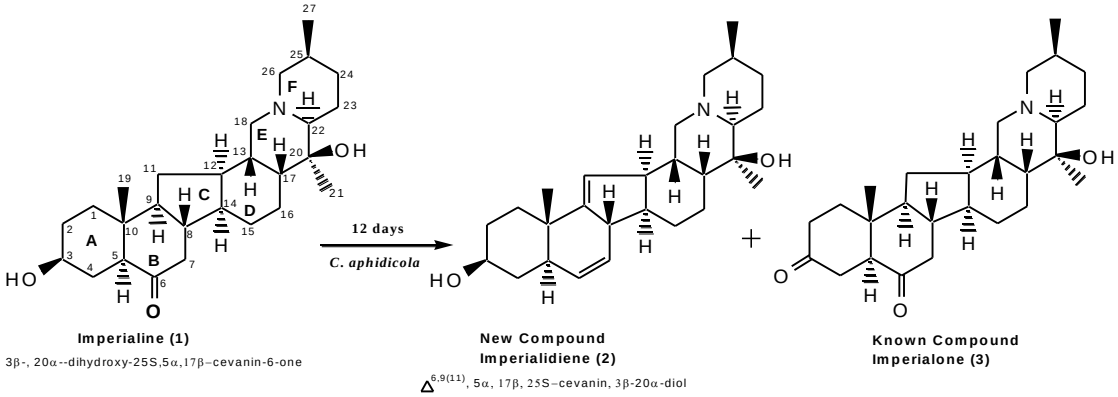
was studied by preparing some easily accessible derivatives through chemical methods[7]–[9]. Microbial transformation of steroids is an extensively studied subject but work on the biotransformation of steroidal alkaloids is rather scarce. The potential of microbial transformations of bioactive natural products to obtain novel chemically inaccessible derivatives prompted us to subject imperialine through this procedure[10], [11]. We report here the microbial transformation of compound 1 by *Cephalosporium aphidicola* to afford two metabolites, imperialidiene (2) and imperialone (**3**) (Figure 1)**.**

Figure 1: Production of Compound 2 and 3 by Microbial Transformation of Imperialine (1)



The fermentation of imperialine (**1**) ($C_{27}H_{43}NO_3$) for 12 days yielded two compounds. The HREIMS of a new compound **2** showed the M^+ at *m/z* 411.3297, thus indicating the molecular formula $C_{27}H_{41}NO_2$ (calcd. 411.3173). The IR spectrum exhibited absorptions at 3413(OH), 2921, 2867(CH_2, CH_3) cm^{-1}. The lack of carbonyl band in the IR spectrum indicated its possible reduction. The ^{1}H-NMR of **2** showed two double doublets at δ 5.15 ($J_{6,7}$ = 9.4 Hz, $J_{6,5}$ = 8.6 Hz) and 5.08($J_{7,6}$ = 9.4 Hz, $J_{7,8}$ = 4.9 Hz) which were assigned to C-6 and C-7 olefinic protons. Reduction of carbonyl group and subsequent dehydration of the resulting alcohol presumably resulted in the formation of a double bond between C-6 and C-7. An olefinic double bond resonating at δ 5.34 could be due to C-11 proton. The unambiguous determination of the position of this olefinic bond was a non-trivial problem, which was eventually solved with the help of COSY-45°, HOHAHA, HMQC and HMBC experiments. The COSY-45° spectrum displayed a cross-peak between H-7 (δ 5.08) and H-8 (δ 1.51). This assignment was further confirmed by HMBC spectrum as it exhibited a cross-peak between H-7 and (δ 5.08) and C-8 (δ 51.2). Another cross-peak in the same spectrum between H-8 (δ 1.51) and the olefinic quaternary carbon at C-9 (δ 140.8) led us to conclude that the later could only be there in the ring C at C-9. A weak

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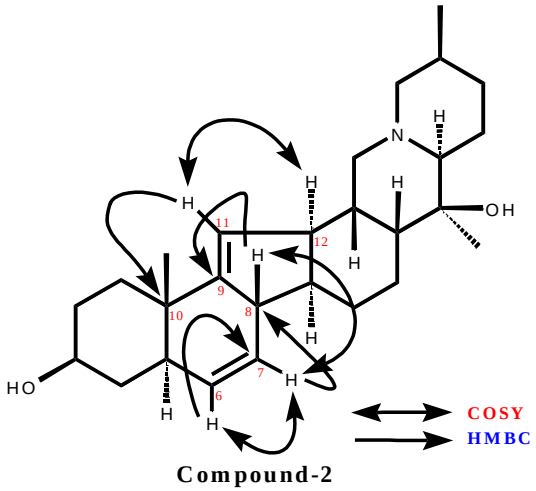
three-bond coupling was also evident in HMBC spectrum between C-10 (δ 35.6) and H-11 (δ 5.34). Moreover there were strong cross-peaks between H-11 (δ 5.34) and H-12 (δ 1.98) in both COSY-45°and TOCSY spectra. The position of the tri substituted double bond was unambiguously assigned in ring C between C-9 and C-11 (Figure 2).

Figure 2: Important COSY (red arrows) and HMBC (blue arrows) Correlations of Compound-2



Compound-2

The second transformed product was found to be a known metabolite of *Petillium raddeuna* and *P. Eduardi*[12]–[14], namely imperialone (**3**). The HR-EIMS of **3** showed the M^+ at *m/z* 427.3118, confirming the molecular formula to be $C_{27}H_{41}NO_3$ (calcd. 427.3066). The ^{13}C NMR showed a signal for an additional carbonyl carbon at δ 209.1. The ^1H-NMR spectrum of **3** features the absence of C-3 αH signal.

EXPERIMENT AND RESULTS

Jasco digital polarimeter (Model DIP-360) was used to record optical rotations in methanol. Hitachi spectrophotometer (Model U-3199) was used to record ultraviolet spectra. Infrared spectra (IR) were measured on a Shimadzu IR 460 spectrophotometer in chloroform. Joel JMS HX 110 mass spectrometer was used to record High Resolution Electron Impact Mass Spectra (HREIMS). Bruker AM-400 and AMX-500 MHz spectrometers were used The NMR spectra. $SiMe_4$ was used as an internal standard while Chemical shifts are in ppm and the coupling constants are reported in Hz. Buchi 535 melting point apparatus were to taken Melting points. The metabolites were purified on flash silica gel. TLC plates (Si gel 60, F_{254}, 0.2 mm E. Merck) pre-coated were used to checked the purity of the samples.

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Fermentation of Imperialine (1) by C. aphidicola: Media for *C. aphidicola* (IMI 68689) was prepared by the reported protocol adjusting the composition of ingredients for 1 L. The imperialine (**1**) (100 mg) was dissolved in ethanol (10 mL) and the resulting solution was evenly distributed among 10 flasks (250 mL each). Each flask was loaded with well-grown cultures of *C. aphidicola*. Fermentation was carried out for 12 days. Positive and negative control flasks were also incubated for the same period. The mycelia were filtered and washed with EtOAc (200 mL). The broth so obtained was extracted with EtOAc (4 L). The organic extract obtained was dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum at 40°C giving a thick brown gum (0.5 g). This was adsorbed on flash silica gel (1.5 g) and chromatographed on a column packed with flash silica (25 g). Hexane-chloroform was used to eluted the column with increasing polarities and then with chloroform-methanol. Compound **3** was obtained on elution with 99.3% $CHCl_3$-0.7 % MeOH, while compound **2** was eluted with 99% $CHCl_3$-1% MeOH.

Imperialidiene (**2**): Colorless amorphous solid (47 mg); $[\alpha]_D^{25}$ -37 (c= 0.0036 in MeOH); UV spectrum (MeOH, λ_{max}, nm) 199; IR spectrum ($CHCl_3$, ν_{max}, cm^{-1}) 3413, 2921, 2867. HREIMS, *m/z* 411.3297 (411.3173 calculated for $C_{27}H_{41}NO_2$). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz) see figure 3(a) to 3(f) and the calculated data are encapsulated in table 1

Figure 3(a): H-NMR spectra of compound 2 (400MHz, CDCL3)

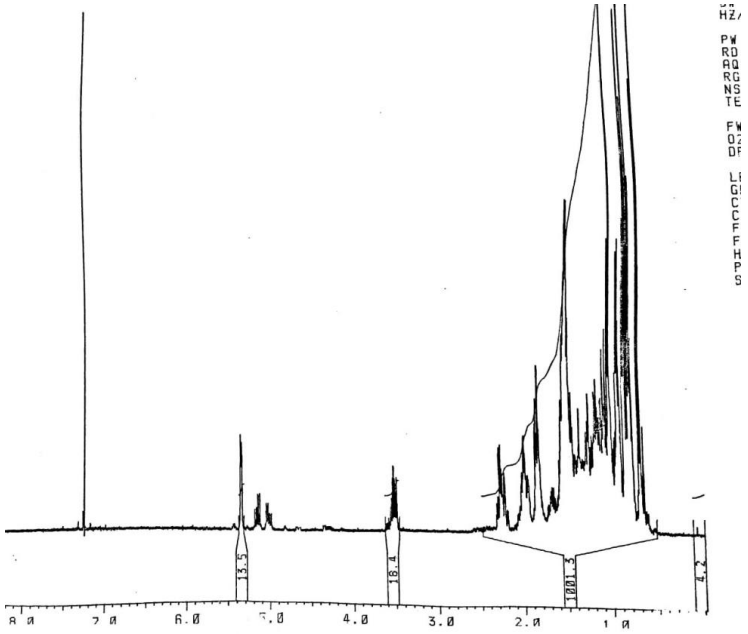

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Figure 3(b): H-NMR spectra of compound 2 (400MHz, CDCL3)

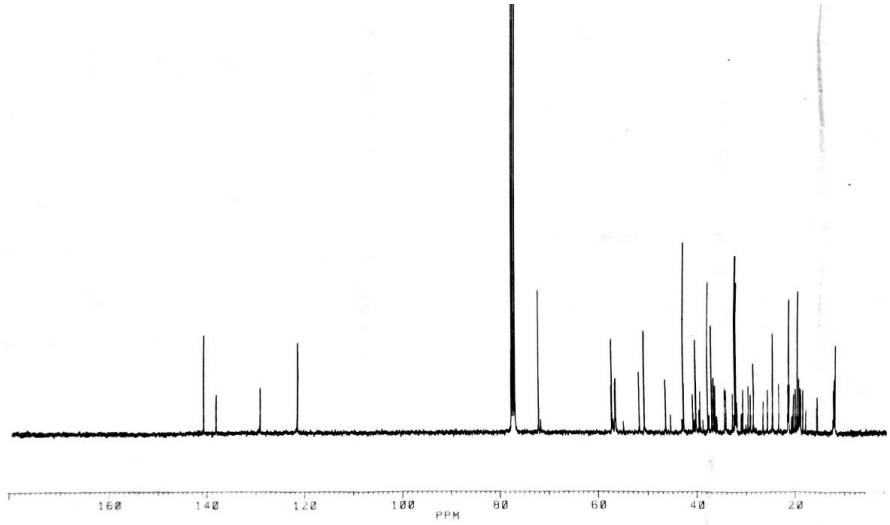

Figure 3(c): HMQC spectrum of compound 2 (CDCL3)

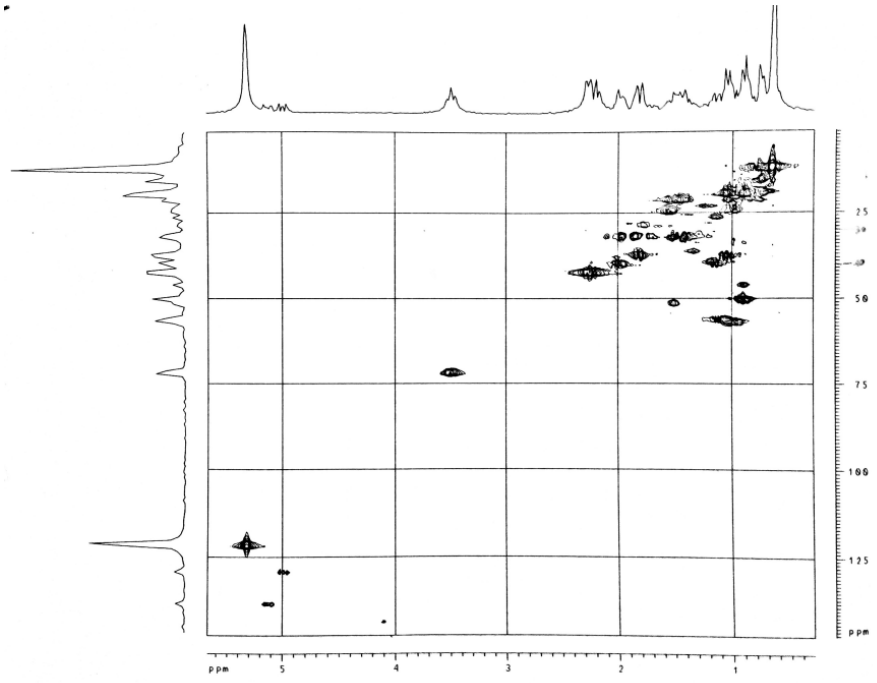

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Figure 3(d): HMQC spectrum of compound 2 (CDCL3)

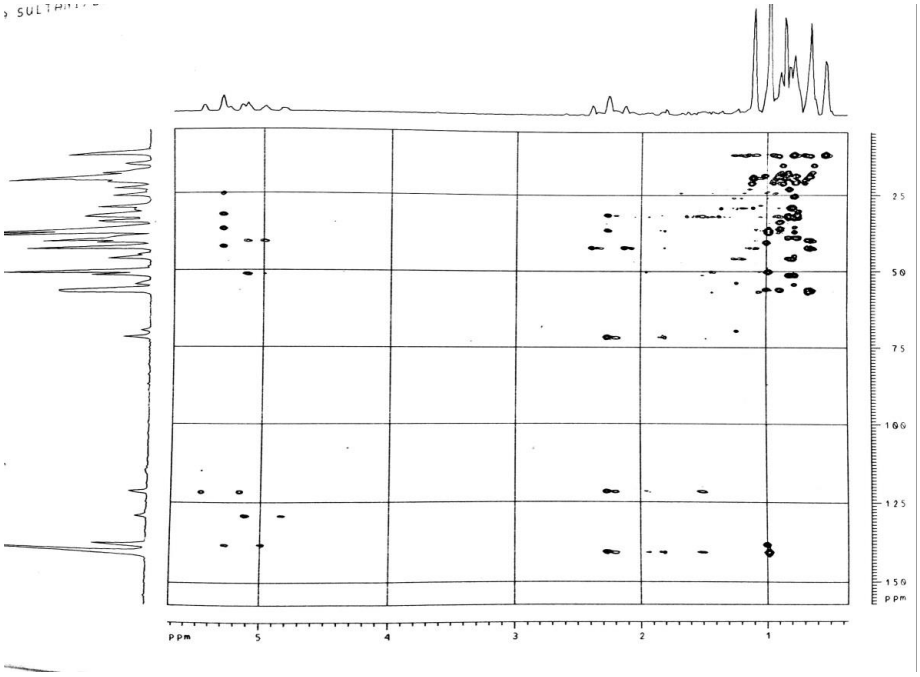

Figure 3(e): HMBC spectrum of compound 2 (CDCL3)

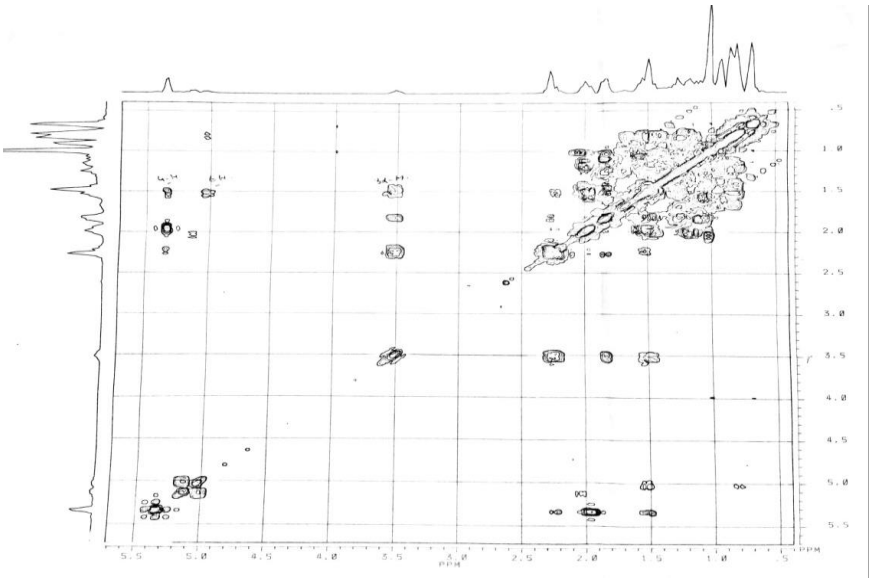

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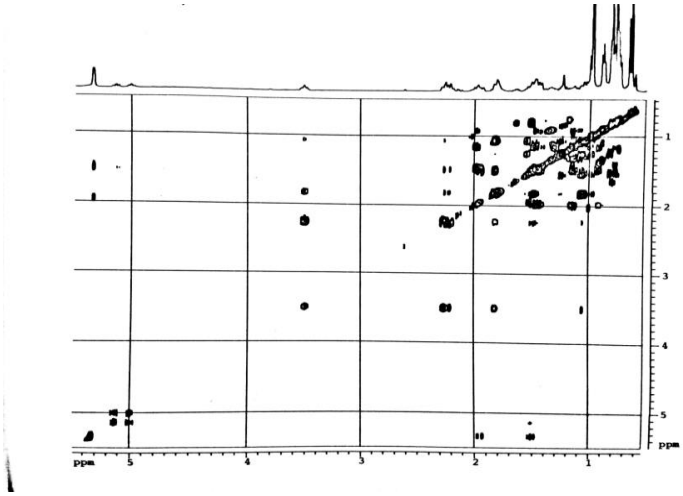
Figure 3(f): HOHAHA spectrum of compound 2 (CDCL3).



Imperialone (**3**)**:** Colorless amorphous solid (20 mg); $[\alpha]_D^{25}$ –29 (c= 0.0035 in MeOH); UV (MeOH) λ_{max} 200 nm; IR ($CHCl_3$) ν_{max}, 2972, 2858; 1706 cm^{-1}: HREIMS *m/z* 427.3118(427.3066 calculated for $C_{27}H_{41}NO_2$). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz) and ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm) see Table-1, for the comprehensive encapsulated calculated values of Table-1 ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm) and ^1H NMR (400 MHz)

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Table 1: **NMR data of compound 2, and 3 (*J* = Hz)**

Carbon No	Chemical shift δ			
	Compound 2		Compound 3	
	^{13}C	^{1}H	^{13}C	^{1}H
1.	37.3	1.81 m	37.4	1.85 m
2.	31.4	1.98 m, 1.85 m	30.3	1.20 m, 1.30 m
3.	71.8	3.52 m ($W_{1/2}$= 19)	209.1	-
4.	28.7	1.81 m	37.0	2.33 m, 2.38 m
5.	56.8	1.01 m	57.5	2.58 dd (*J*= 5.8, 3.2)
6.	138.3	5.15 dd (*J*= 9.4, 8.5)	211.2	-
7.	129.4	5.08 dd (*J*= 9.4, 8.0)	38.1	2.10 m
8.	51.2	1.51 m	36.05	2.57 m
9.	140.8	-	53.5	1.33 m
10.	35.6	-	35.5	-
11.	121.7	5.34 d (*J*= 5.3)	28.0	1.88 m
12.	40.4	1.98 m	32.14	1.38 m
13.	35.9	1.34 m	31.9	1.55 m
14.	56.2	1.13 m	35.8	1.37 m
15.	23.2	1.32 m	26.1	1.18 m
16.	21.4	1.48 m	21.7	1.42 m
17.	45.9	0.90 m	50.9	3.48 m
18.	42.4	2.40 m, 2.18 m	46.6	2.38 m
19.	12.0	0.68 s	12.0	0.68 s
20.	71.4	-	77.2	-
21.	19.4	0.99 s	19.0	0.99 s
22.	56.9	0.90 m	56.6	1.23 m
23.	24.3	1.59 m	23.1	1.25 m
24.	32.0	1.40 m, 1.50 m	29.7	1.27 m
25.	38.9	1.15 m	29.1	1.30 m
26.	39.8	1.92 m, 2.00 m	56.1	1.15 m
27.	21.0	1.01 d (*J*= 6.7)	15.4	0.78 d (*J*= 7.9)

CONCLUSION

Microbial transformation of bioactive natural product Imperialine (**1**) by using the fungus *Cephalosporium aphidicola* yielded a new and a known compound. These compounds were fully characterized by using spectroscopic techniques and identified as Imperialidiene (**2**), and Imperialone (**3**) respectively.

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