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# Antifungal Natural Products from Medicinal Plants of Pakistan*

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A number of diseases in humans, animals, insects and plants are caused by pathogenic fungi. Several fungi also produce toxins in foods and can cause poisoning. Antifungal compounds are therefore important both for controlling serious fungal diseases of animals and plants, and for preserving food and other organic materials against attack by fungi. Our work on antifungal phytochemicals of medicinal plants has resulted in the isolation of a number of interesting compounds. The agar diffusion method was used to determine the antifungal activity of the pure isolates and extracts against a number of highly pathogenic fungi. The structures of the antifungal principles were determined by using modern spectroscopic and/or X-ray diffraction techniques.

## Aryltetralin Lignans from the Leaves of Podophyllum Hexandrum Royle

A number of lignans isolated from *Podophyllum* species have shown in a wide range of biological activities such as antitumor, antimitotic and antiviral activities. Some of them have also shown toxicity to fungi, insects and vertebrates[1,2]. The successful chemical conversions of the major constituent podophyllotoxin into the clinically useful anticancer drugs etoposide and teniposide has also triggered further research in this area[3].

Our investigations on *P. hexandrum* Royle collected from Muzaffarabad, Pakistan, have resulted in the isolation of two new aryltetralin lignans, 4'-*O*-demethyldehydropodophyllotoxin (**1**) and picropodophyllone (**2**) earlier reported as semisynthetic products. Compounds **1** and **2** have shown strong antifungal activity against *Epidermophyton floccosum, Curvularia lunata, Nigrospora oryzae, Microsporum canis, Allescheria boydii* and *Pleuretus ostreatus,* while compounds **2** and also shown activity against *Drechslera rostrata* as determined by the agar diffusion method[4].

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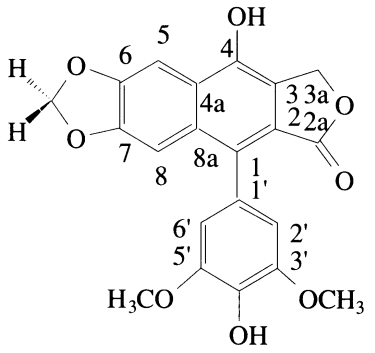
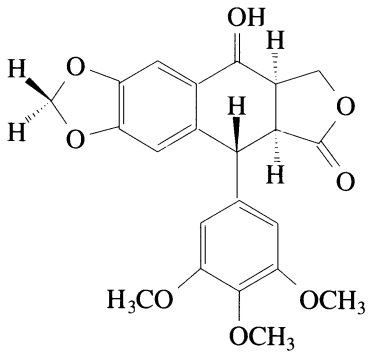



(1)




(2)

Compound **1** was isolated as an amorphous powder. The molecular formula $C_{21}H_{16}O_8$ was obtained from the high resolution electron impact (HREI) mass measurement of the molecular ion (*m/z* 396.0799) indicating the presence of 14 double bond equivalents in the molecule. The $M^+$ was further confirmed by +ve FAB mass spectrometry. The overall spectral behaviour of **1** was similar to that reported for the semisynthetic product, 4'-*O*-demethyldehydropodophyllotoxin[5]. The UV spectrum exhibited absorption bands at 205, 229, 264, 314 and 357 nm, characteristic of a tetradehydropodophyllotoxin lignan nucleus[6]. The absorption bands in the IR spectrum at 3437, 2900, 1742, 1609 and 1448 and 1029 $cm^{-1}$ indicated the presence of phenolic OH, C-H, lactone carbonyl, aromatic C=C and C-O functionalities, respectively.

The $^{13}$C-NMR spectra (broad-band decoupled and DEPT)[7] showed 16 signals representing 19 carbons. An examination of the structure (**1**) ultimately deduced for the compound shown that the aromatic ring substituted at C-1 contains three pairs of identical carbons (the two -$OCH_3$ carbons appearing at $\delta$ 56.3, the two carbons and which the -$OCH_3$ groups are attached resonating at $\delta$ 148.7 and the two carbons *ortho* to the methoxy group resonating at $\delta$ 109.3). The signals for the C-1' and C-4' quaternary carbons were too weak to be detected in the $^{13}$C-NMR spectrum. An ester carbonyl carbon resonated at $\delta$ 170.0, a methylenedioxy carbon appeared at $\delta$ 102.1 and a downfield methylene carbon resonated at $\delta$ 66.9, while the remaining 13 signals appeared between $\delta$ 100 - 150, indicating a substituted aromatic system.

In the $^1$H-NMR spectrum of **1**, a 6H single resonated at $\delta$ 3.81 representing two -$OCH_3$ groups in an identical magnetic environment. A downfield methylene singlet at $\delta$ 6.11 was characteristic of a methylenedioxy group and its appearance as a singlet was indicative of the lack of chirality in the molecule. Another 2H downfield singlet at $\delta$ 5.50 was due to the methylene protons (C-3H) sandwiched between an oxygen function and the quaternary carbon. A 2H singlet in the aromatic region of the spectrum at $\delta$ 6.97 was assigned to the two aromatic protons in an identical magnetic environment. *i.e.* the C-2' and C-6' protons. Two more downfield signals appeared as broad singlets at $\delta$ 8.14 and 7.46 and were ascribed to the two remaining aromatic protons of the skeleton. The absence of coupling interactions between these aromatic protons indicated their *para* disposition and they were therefore assigned to the C-5 and C-8 protons respectively.

The *H*eteronuclear *M*ultiple *Q*uantum *C*oherence (HMQC) experiment[8] was performed to establish connectivities between the protons and their respective carbons. Hence, the C-5 and C-8 carbons ($\delta$ 99.0 and 104.0) in the aromatic moiety displayed one-bond interactions with the protons resonating at $\delta$ 8.14 and 7.46 respectively, while another set of carbons resonating at $\delta$ 109.3 (C-2' and C-6') in ring D showed correlation with the C-2' and C-6' protons ($\delta$ 6.97). The C-3a methylenic protons exhibited heteronuclear couplings with the C-3a carbon ($\delta$ 66.9). The *H*eteronuclear *M*ultiple *B*ond *C*onnectivity (HMBC) experiment (9) was used for the unambiguous chemical shift assignments. Thus the long-range interactions between the protons

at $\delta$ 6.97 (C-2'H and C-6'H) with the carbons resonating at $\delta$ 148.7 (C-5') and $\delta$ 137.4 (C-4') suggested that they are part of ring D. The aromatic C-5 proton resonating at $\delta$ 8.14 exhibited long-range interactions with the carbons at $\delta$ 148.9 (C-7) and 132.5 (C-4a). The C-3a methylene protons displayed HMBC interactions with C-3 ($\delta$ 120.0), while C-8H showed interactions with C-7, C-8a and C-1. The mass spectrum included peaks at $m/z$ 396 ($M^+$), 353, 334, 281 and 139. This spectroscopic data unambiguously defined that **1** is the naturally occurring 4'-*O*-demethyl derivative of dehydropodophyllotoxin. Compound **1** exhibited some cytotoxic and antitumour activity as reported in the literature [5].

Picropodophyllone (**2**), a $1R, 2S, 3R$ isomer of podophyllotoxone, has also been known synthetically for several years, but has not previously been reported as a natural product[10]. The molecular formula $C_{22}H_{20}O_8$ of **2** was again determined by HREI MS ($m/z$ 412.1138). The UV spectrum of **2** displayed absorption bands at 206, 240, 280 and 324 nm characteristic of the podophyllotoxone skeleton[11]. The infrared spectrum contained bands at 2839 (C-H), 1772 (lactone carbonyl), 1661 (ketone carbonyl), 1584 (C=C) and 1125 (C-O) $cm^{-1}$.

The $^{13}$C-NMR spectra (broad-band decoupled and DEPT) contained a lactone carbonyl signal at $\delta$ 175.5 and an $\alpha,\beta$-unsaturated ketonic resonance at $\delta$ 193.4. The three methoxy carbons appeared at $\delta$ 56.3 ($2\times OCH_3$) and $\delta$60.8($-OCH_3$). The methylenedioxy carbon yielded a characteristic signal at $\delta$ 102.2 A downfield methylene signal at $\delta$ 70.5 was assigned to the C-3a methylene carbon containing an oxygen function. The methine carbons resonated at $\delta$ 43.5, 46.7 and 43.4 representing the carbon atoms of ring B. The aromatic carbons appeared in two groups. The signals between $\delta$ 104-107 were due to unsubstituted aromatic carbons while the signals resonating between $\delta$ 127-154 represented either oxygen-bearing or quaternary aromatic carbons. Only 17 signals were visible in the $^{13}$C-NMR spectra representing 22 carbons.

The $^1$H-NMR spectrum of **2** contained a 6H and a 3H singlets at $\delta$ 3.73 and 3.77 which could be assigned to the three methoxy groups, two of which have identical magnetic environment. The methylenedioxy protons appeared as two AB doublets at $\delta$ 6.02 and 6.01 indicating the presence of chirality in the molecule. Similarly a set of geminally coupled protons, resonating at $\delta$ 4.73 as a doublet and $\delta$ 4.32 as a multiplet, represented methylene protons *i.e.* C-3a protons sandwiched between an oxygen and methine.

The COSY 45° spectrum[12] displayed cross-peaks between the signals at $\delta$ 4.73 and 4.32 due to their geminal disposition, while the cross-peak between $\delta$ 4.32 and 3.28 represented vicinal coupling between one of the methylenic protons with the neighbouring methine proton (C-3H). The spin system comprising C-3a, C-3, C-2 and C-1 in picropodophyllone (**2**) was further investigated by HOHAHA experiments[13] recorded with mixing intervals of 20, 60 and 100 ms. Thus the C-3a $\alpha$ and $\beta$ protons at $\delta$ 4.73 and 4.32 showed geminal coupling and also HOHAHA interactions with the C-3 and C-2 protons ($\delta$ 3.28).

The HMQC spectrum of **2** showed that the most downfield proton resonating at $\delta$ 7.47 had a one-bond heteronuclear interaction with the C-5 carbon ($\delta$ 106.0). The C-8 proton ($\delta$ 6.66) showed shift correlation with its respective carbon resonating at $\delta$ 109.4. The methylenic C-3a $\alpha$ and $\beta$ protons ($\delta$ 4.32 and 4.73). displayed connectivities with the C-3a carbon ($\delta$ 70.5). In the HMBC spectrum, the proton resonating at $\delta$ 4.67 (C-1H) showed long-range interactions with C-8a, C-4a, C-3, C-2 and also with the C-2' and C-6' carbons in ring D. Similarly the C-5 proton ($\delta$ 7.47) in the aromatic moiety exhibited interactions with C-6, C-7 and C-8a, while the aromatic proton resonating at $\delta$ 6.66 (C-8H) showed interaction with C-4a, C-6 and C-7.

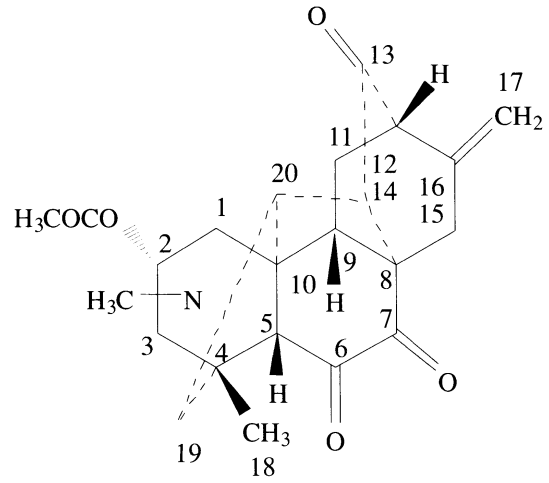
The EI MS of **2** displayed the $M^+$ peak at $m/z$ 412.1138 ($C_{22}H_{20}O_8$). The ion at $m/z$ 367 ($M^+$-$HCO_2$) represented the fragment $C_{21}H_{19}O_6$. The peaks at $m/z$ 167 ($M^+$-245) and 200 ($M^+$ -212) were due to fragments $C_9H_{12}O_3$ and $C_{12}H_8O_3$ respectively. Other peaks at $m/z$ 353, 337, 297, 227 139, etc.

were also in complete agreement with the structure **2**. Complete analysis of the $^{1}$H-NMR and $^{13}$C-NMR spectra with the aid of COSY, HMQC and HMBC experiments led to the elucidation of the structure of the compounds as shown in structure **2**.

## Diterpenoid Alkaloids From Delphinium Denudatum Wall

*Delphinium* species (Ranunculaceae) contain diterpenoid alkaloids which are generally of the veatchine or atisine-type[14]. *Delphinium* species have also been used for the treatment of itches and other skin eruptions in folklore. *Delphinium denudatum* Wall is extensively found in the Western Himalayas and in Kashmir at altitudes, of 8,000 to 12,000 ft. especially on grassy slopes. The roots of this plant are bitter and considered stimulant, alterative and tonic[15]. Organic extracts of the plant have been shown to have antimicrobial and immunomodulating properties[16]. The radioprotectant effect of the aqueous extract of *D. denudatum* against radiation-induced changes in rat myocardium has also been investigated[17].

The ethanolic extracts of the roots of *D. denudatum* collected from Kashmir (Pakistan) have shown antifungal activity[4] against *Stachybotrys atra, Trichophyton longifusus, Curvularia lunata, Drechslera rostrata, Epidermophyton floccosum, Microsporum canis, Nigrospora oryzae,* and *Ganoderma applanatum,* and antibacterial activity[4,18] against *Corynebacterium diptheriae, Proteus vulgaris, Salmonella typhi* and *Klebsiella pneumoniae.* Using bioassay-directed isolation stagety we recently isolated two new antifungal diterpenoid alkaloids, delphatambine (**3**) and 8-acetylheterophyllisine (**4**), from the roots of *Delphinium denudatum* Wall. Both compounds showed antifungal against *Allescheria boydii, Epidermophyton floccosum* and *Aspergillus niger.*

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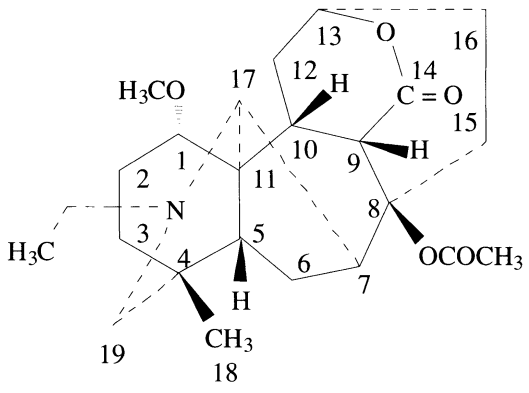



(4)

Delphatambine (**3**), an atisine-type diterpenoid alkaloid was isolated from the roots of *D. denudatum* by column and thin-layer chromatography. The HREI mass spectrum showed the molecular ion at $m/z$ 397.1882 corresponding to the molecular formula, $C_{23}H_{27}NO_5$ which required eleven degrees of unsaturation. Compound **3** exhibited strong UV absorption at 301 nm. The IR spectrum of the compound exhibited absorption bands at 1720 (ester C=O), 1690 (ketone C=O) and 1597 (C=C) cm$^{-1}$. The $^{1}$H-NMR spectrum of **3** exhibited three three-proton singlets at $\delta$ 1.41, 2.06 and 2.20 which showed $^{1}$H/$^{13}$C interactions in the HMQC[8] spectrum with the carbons resonating at $\delta$ 31.0, 21.8 and 41.9 and were therefore assigned to the 18-$CH_3$, $OCOCH_3$ and $NCH_3$ protons. Two downfield one-proton triplets at $\delta$ 4.93 and 5.04 showed one-bond coupling in the HMQC spectrum withthe carbon at $\delta$ 112.7 and were accordingly assigned to the

C-17 olefinic protons. The C-17 olefinic protons at $\delta$ 4.93 showed COSY connectivities with the C-12 proton at $\delta$ 2.99 as well as with the C-15 proton at $\delta$ 2.52 and 3.12 due to allylic coupling. Both the C-17 protons appeared as two triplets at $\delta$ 4.93 and 5.04 showing geminal and allylic couplings of about 2 Hz. The C-15 proton at $\delta$ 2.52 resonated as a doublet of triplets, showing geminal coupling of 18.2 Hz, and vicinal coupling with the C-17 olefinic protons of 2.7 Hz. The C-5H appeared as a 1H singlet at $\delta$ 2.90 showing one-bond $J_{CH}$ interaction in the HMQC spectrum with the carbon at $\delta$ 53.7, its downfield value indicating the presence of an adjacent electron-withdrawing carbonyl. The one-proton downfield multiplet at $\delta$ 5.24, assigned to the methine proton geminal to the acetate group, was found to be coupled in the COSY spectrum to two different sets of methylene protons at $\delta$ 1.65 and 1.89 (C-1 $CH_2$) and $\delta$ 1.67 and 1.95 (C-3 $CH_2$). This indicated the presence of a methine at C-2. An isolated AB double doublet at $\delta$ 2.43 and 2.85 was assigned to the C-19 $CH_2$ protons. The long-range $^1H/^{13}C$ shift correlations were determined through the HMBC experiment[8].

The $^{13}$C-NMR spectra (DEPT, BB) showed the presence of four carbonyl groups. Three downfield signals resonating at $\delta$ 205.0, 192.0 and 208.0 were assigned to the ketonic carbonyl carbons, while the ester carbonyl resonated at $\delta$ 165.0. Two olefinic signals at $\delta$ 140.0 and 112.7 were assigned to the C-16 and C-17 carbons respectively.

The structure of delphatambine (**3**) was unambiguously solved by X-ray diffraction technique. Suitable crystal formed in the orthorhombic space group $P2_12_12_1$ with $a$ = 8.043(4), $b$ = 13.695(7) and $c$ = 18.297(10) Åwas selected for the diffraction study. One molecule of composition $C_{23}H_{27}NO_5$ formed the asymmetric unit. All unique diffraction maxima with $2\theta \leq 105°$ were collected using $2\theta : \theta$ scans and graphite monochromated CuK$\alpha$ radiation (1.54178 Å). A total of 1574 unique reflections were collected, and 1485 (94%) were judged observed ($|F_o| \geq 6\delta(|F_o|)$) and used in subsequent calculations. The structure was phased using direct methods and refined using full-matrix least-squares with anisotropic heavy atoms and isotropic riding hydrogens to a conventional crystallographic residual of 0.051 ($R_w$ = 0.0727) for the observed data. A computer-generated drawing of the final X-ray model of delphatambine is given in Figure 1. Hydrogens are omitted for clarity and no absolute configuration is implied[20,21].

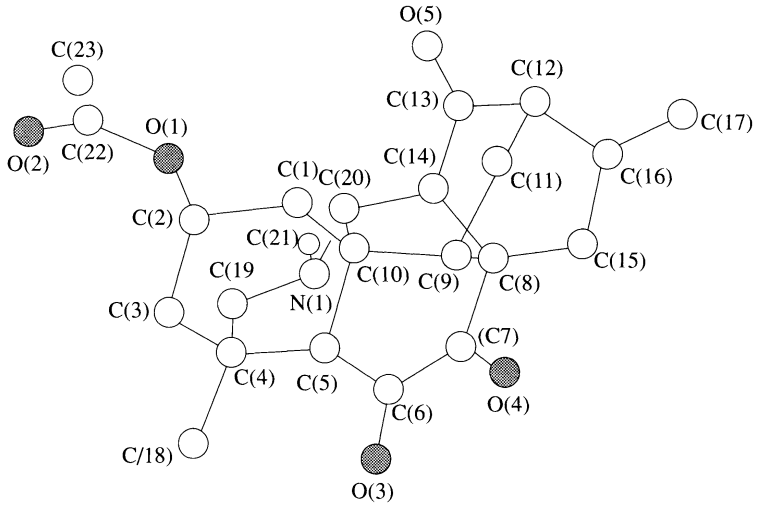



**Figure 1.** A computer-generated perspective drawing of the final X-ray model of delphatambine (**3**).

## Withanolide from *Withania Coagulance* Dun.

Withanolides are $C_{28}$ steroidal lactones which have been isolated from different plants of the family Solanaceae as *Withania somnifera, W. coagulance, Acnistus australis* and *Datura metel.* Plants of genus

*Withania* are known to exhibit a variety of pharmacological activities mainly due to the presence of withanolides[22–25]. We have isolated and carried out the structure elucidation of a new antifungal withanolide, 17$\beta$-hydroxywithanolide K(**5**), from this plant.

The antifungal activity of the crude extract and pure 17$\beta$-hydroxywithanolide K (**5**) was tested against nine highly pathogenic fungal isolates *i.e. N. oryzae, A. niger, C. lanata, P. ostreatus, S. atra, A. boydii, D. rostrata, M. canis* and *E. floccosum.* Both the crude extract and 17$\beta$-hydroxywithanolide K(**5**) showed good activity against all the above mentioned organisms. Minimum inhibitory concentrations (MIC) of 17$\beta$-hydroxywithanolide K (**5**) against all the above tested organisms are found to be of the order of 300 $\mu$g/ml.

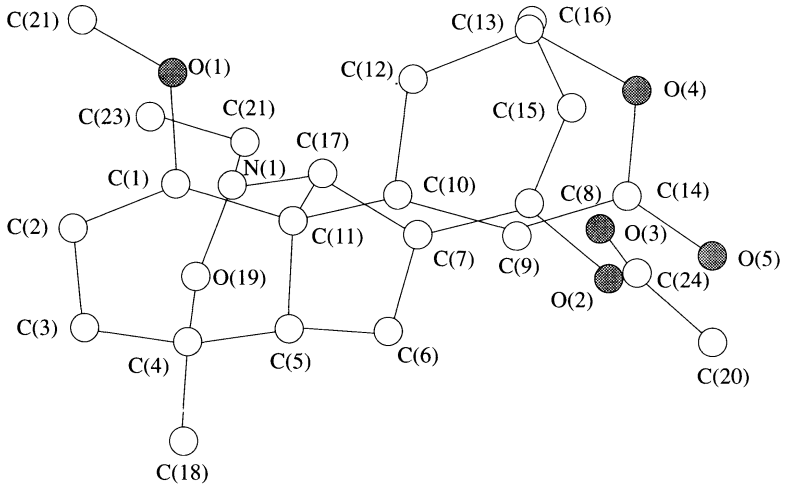



**Figure 2.** A computer-generated perspective drawing of the final X-ray model of 8-acetylheterophyllisine (**4**).

The EI MS of 17$\beta$-hydroxywithanolide K (**5**) afforded the $M^+$ at $m/z$ 470.2677 corresponding to the formula $C_{28}H_{38}O_6$, indicating ten degrees of unsaturation. The presence of a hydroxyl, a ketonic carbonyl and an $\alpha,\beta$-unsaturated lactone was indicated by the IR absorption at 3420, 1690 and 1675 $cm^{-1}$. The presence of an $\alpha,\beta$-unsaturated lactone was indicated by the UV absorption at 223 nm.

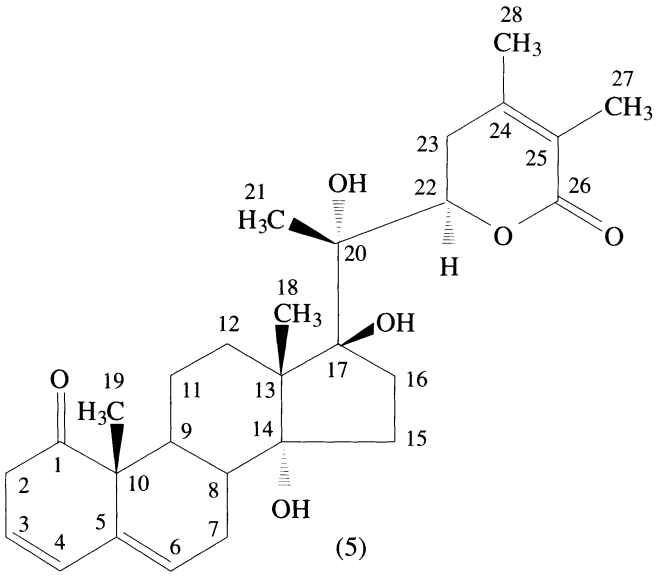
The $^1$H-NMR spectrum (500 MHz, $CDCl_3$ + $CDCl_3$ + $CD_3OD$) of 17$\beta$-hydroxywithanolide K (**5**) showed five tertiary methyl resonating at $\delta$ 1.02, 1.19, 1.32, 1.78 and 1.86. Two mutually coupled olefinic signals resonating at $\delta$ 5.55 (m) and 6.01 (dd, $J_{4,3}$ = 9.6 Hz, $J_{4,22}$ = 2.3 Hz) were assigned to the C-3 and C-4 vinylic protons respectively. Another vinylic signal resonating at $\delta$ 5.71 (dd, $J_{6,7b}$ = 4.9 Hz, $J_{6,7a}$ = 2.1 Hz) was assigned to H-6 of the conjugated diene. A downfield methine double doublet at $\delta$ 4.84 ($J_{22,23a}$ = 12.2 Hz, $J_{22,23b}$ = 4.2 Hz) was assigned to the C-22 proton of the lactone moiety.

The HREI mass spectrum of **5** showed the molecular ion at $m/z$ 470.2670. The base peak at $m/z$ 125 arose by the cleavage of the C-20/C-22 bond and confirmed the presence of a lactone moiety. The ion at $m/z$ 169 resulted from the cleavage of the C-17/C-20 bond.

The $^{13}$C-NMR spectrum (125 MHz, $CDCl_3$ + $CD_3OD$) of **5** showed resonances for all 28 carbons and assignments were made by comparison with known withanolides. The $^1$H/$^{13}$C long-range coupling information was obtained from the inverse heteronuclear multiple bond connectivity (HMBC) experiment. The methine proton at $\delta$ 6.01 correlated with C-1 ($\delta$ 196.6), C-5 ($\delta$ 140.1) and C-2 ($\delta$ 39.2). Another methine proton at $\delta$ 5.71 showed long range couplings with C-2 ($\delta$ 39.2), C-10 ($\delta$ 52.3), C-3 ($\delta$ 127.1) and C-5 ($\delta$ 140.1). The H-22 methine proton correlated with C-21 ($\delta$ 18.3), C-17 ($\delta$ 87.0), C-24 ($\delta$ 151.7) and C-20 ($\delta$ 78.2).

In the COSY 45° spectrum the downfield signal resonating at $\delta$ 5.56 (m) showed vicinal coupling with the C-2 protons at $\delta$ 3.23 (m) and 2.71 (m), and the C-4 proton at $\delta$ 6.01 (dd). The proton at C-6 showed

COSY 45° cross-peaks with the C-4 proton at $\delta$ 6.01 and C-7 methylene protons at $\delta$ 2.60 (mm) and 2.10 (m). The C-22 proton also showed a cross-peaks with the C-23 methylene protons ($\delta$ 2.55 and 2.45). These spectroscopic studies led to structure **5** for the new antifungal 17$\beta$-hydroxywithanolide K. Compound **5** was obtained earlier by partial derivatization of withanolide F by Velde *et al.* but has not been isolated from natural sources[26].

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### References

1. W. Dymock, "Pharmacographia Indica", republished by the Institute of Health and Tibbi Research under the auspices of Hamdard National Foundation, Pakistan, *1* **69,** (1972).
2. W.D. Macrae, and G.H.N. Towers, **Phytochemistry, 23,** 1207 (1984).
3. B.F. Issel, F.M. Muggia, S.K. Carter, Etoposide (**VP**-16)-Current Status and New Development, Academic Press, Orlando (1984).
4. J.D. Paxton, in "Methods in Plant Biochemistry", vol. 6, pp. 33; Academic Press, London (1991).
5. S.A. Beers, Y. Imakura, H.J. Dai, D.H. Li, Y.C. Cheng, and K.H. Lee, **J. Nat. Prod., 51,** 901 (1988).
6. M. Tanoguchi, M. Arimoto, H. Saiki, and H. Yamaguchi, **Chem. Pharm. Bull., 35,** 4162 (1987).
7. D.M. Doddrell, D.T. Pegg, and M.R. Bendall, **J. Magn. Res., 48,** 323 (1982).
8. A. Bax, and S. Subramanian, **J. Magn. Res., 67,** 565 (1986).
9. A. Bax, and M.F. Summers, **J. Am. Chem. Soc., 108,** 2093 (1986).
10. W.J. Gensler, F. Johnson, and A.D.B. Sloan, **J. Am. Chem. Soc., 82,** 6074 (1960).
11. P.M. Dewick, and D.E. Jackson, **Phytochemistry, 20,** 2277 (1981).

12. Atta-urRahman, "One- and Two-Dimensional NMR Spectroscopy" Elsevier Science Publishers, Amsterdam, 269 (1989).
13. L. Braunshweiler, and R.R. Ernst, **J. Magn. Res., 53,** 521 (1983).
14. Atta-ur-Rahman, "Handbook of Natural Products Data, Diterpenoid and Steroidal Alkaloids", Elsevier Science Publishers, Amsterdam (1990).
15. S.S. Bhatnager, "The Wealth of India", C. S. I. R., New Delhi, India (1952).
16. R. Siddiqui, S. Kazmi, and S. Shekhani, **"Abstracts of the Annual Meetings of the American Society for Microbiology", 90,** 384 (1990).
17. A.B. Khan, **J. Mol. Cell. Card., 21,** S171 (1989).
18. R. Carran, A. Maran, J.M. Mentero, L. Fernandozlago, and A. Domingnez, Plants Medicinnales **Phytotherapie, 21,** 195 (1987); **21,** 195 (1987).
19. A, Bax, **J. Mag., Reson., 53,** 517 (1984).
20. R.G. Sheldrick, "SHELXTL PLUS, Structure Solving Package", Nicolet (now Siemens), Madison (1986).
21. Archival crystallographic data have been deposited with teh Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1 EW, U. K.
22. K.N. Gaind, and R.D. Budhiraja, **Ind. J. Pharm., 29,** 185 (1967).
23. R.D. Budhiraja, S. Sindhi, and K.N. Gaind, **Ind. J. Physiol Pharmacol., 27,** 129 (1983).
24. Atta-ur-Rahman, S. Abbas, Dure-e-Shahwar, S.A. Jamal, and M.I. Choudhary, **J. Nat. Prod., 56,** 1000 (1983).
25. S.M. Kupchan, W.K. Anderson, P. Bollinger, R.W. Doskotch, and R.M. Smith, **J. Org. Chem., 34,** 3858 (1969).
26. V. Van de Velde, D. Lavie, R.O. Budhiraja, S. Sudhir, and K.N. Grag, **Phytochemistry, 22**, 2253 (1983).