Balanol: A Novel and Potent Inhibitor of Protein Kinase C from the Fungus *Verticillium balanoides*

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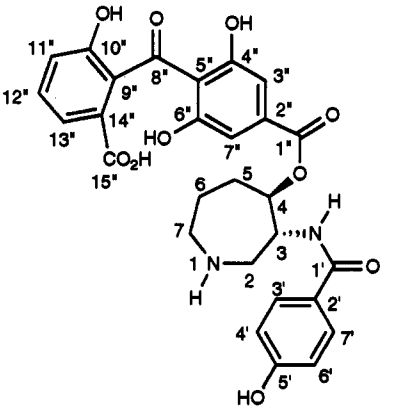
Protein kinase C (PKC), a family of serine/threonine-specific kinases, plays a key role in cell growth, metabolism, and differentiation.[1] Because the activated enzyme has been implicated in several disease processes, namely, cancer, inflammation, cardiovascular dysfunctions, diabetic complications, asthma, central nervous system disorders, and HIV infection, agents that inhibit PKC may prove to be of therapeutic value.[2] In our search for novel and potent PKC inhibitors, we discovered balanol, an unusal metabolite produced by the fungus *Verticillium balanoides*. In this communication we describe the isolation, structure, and absolute stereochemistry of balanol. Balanol inhibits PKC in the low nanomolar concentrations.

V. balanoides collected from *Pinus palustris* needle litter near Hoffman, NC, was cultured in yeast extract, peptone, dextrose, malt, and cornmeal media. The freeze-dried culture was repeatedly extracted with MeOH, and the organic extract (IC_{50} 10–20 μG/mL against PKC) was partitioned between *n*-BuOH and H_2O. Bioassay-guided fractionation of the *n*-BuOH-soluble material ($IC_{50} < 1$ μG/mL) by gel permeation on Sephadex LH-20 with a CH_2Cl_2–MeOH gradient followed by reversed-phase (ODS silica) HPLC furnished pure balanol (**1**) as a yellow amorphous solid, $[\alpha]_D$ −129° (*c* 0.25, MeOH). Approximately 12 mg of balanol was obtained from 40 L of culture. The molecular formula was established as $C_{28}H_{26}N_2O_{10}$ by HR-FABMS ($[M + H]^+$, *m/z* 551.1685, Δ 2.7 mmu). The IR spectrum indicated the presence of hydroxyl, ester, and amide functionalities. Detailed analysis of ^1H, ^{13}C (Table I), and 2D NMR (COSY, NOESY, TOCSY, HMQC, and HMBC) data enabled us to propose structure **1** for balanol and unambiguously assign all the protons and carbons. The main core of balanol was identified to be a hexahydroazepine ring with amide and ester substituents at C-3 and C-4, respectively, by ^1H–^1H decoupling and TOCSY experiments. The ^1H NMR spectrum supported the presence of 1,4-disubstituted (δ_H 7.62, 6.74, each 2H, d, J = 8.7 Hz), 1,2,6-trisubstituted (δ_H 6.64, brd, 7.07, t, 6.96, brd, each 1H, J = 7.6 Hz), and 1,3,4,5-tetrasubstituted (δ_H 6.66, 2H, s) benzene rings. On the basis of the HMBC experiment, as well



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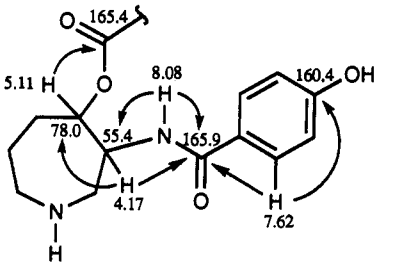
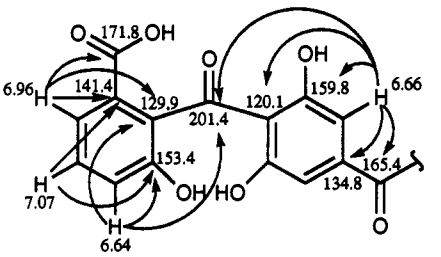
as ^1H and ^{13}C NMR data, the 1,4-disubstituted benzene ring was identified to be *p*-hydroxybenzoyl and attached to the nitrogen, which in turn adjoined the C-3 of the hexahydroazepine ring as depicted in **a**. Accordingly, H-3 (δ_H 4.17), the amide proton (δ_H



a



b

8.08), and H-3′,7′ (δ_H 7.62) all showed HMBC cross peaks to the amide carbonyl (δ_C 165.9). Additional proof for this assignment was obtained from the basic hydrolysis (K_2CO_3–H_2O–MeOH) of **1**, which produced the alcohol **2**. The remaining two substituted benzene rings were linked *via* a carbonyl (δ_C 201.4), and the C–C connectivities were established by HMBC experiment as shown in **b**. The significant HMBC correlation which led to the assignment of the tetrasubstituted benzene ring attached to C-4 of the hexahydroazepine ring through an ester linkage was evident from the cross peaks shown by H-4, H-3″, and H-7″ (degenerate) to the ester carbonyl (δ_C 165.4).[3] The relative stereochemistry at C-3 and C-4 was assigned *trans* on the basis of the observed ^1H–^1H coupling constant ($J_{3,4}$ = 7.6 Hz) and NOE data. Significant NOE interactions, as depicted in **c**, were observed between the amide proton and H-3′, H-2b, and H-4 and between H-4 and H-2b. Proton H-2b showed large coupling ($J_{2b,3}$ = 7

(1) (a) Nishizuka, Y. *Nature* **1988**, *334*, 661–665. (b) Parker, P. J.; Kour, G.; Marais, R. M.; Mitchell, F.; Pears, C.; Schaap, D.; Stabel, S.; Webster, C. *Mol. Cell. Endocrinol.* **1989**, *65*, 1–11. (c) Stabel, S.; Parker, P. J. *Pharmacol. Ther.* **1991**, *51*, 71–95.

(2) Bradshaw, D.; Hill, C. H.; Nixon, J. S.; Wilkinson, S. E. *Agents Actions* **1993**, *38*, 135–147.

(3) The alternate assignment of the phenolic groups, i.e., on C-3″ and C-7″ instead of C-4″ and C-6″ of the tetrasubstituted benzene ring, however, remained a possibility. The assignment shown in the formula is favored on the basis of the fact that the substituted benzophenone of this type is biogenetically derived from its respective anthraquinone precursor.[4] In addition, the carbon shifts also favored the assignment of the phenolic groups on C-4″ and C-6″ as shown in **1**.

(4) Turner, V. R.; Aldridge, D. C. *Fungal Metabolites II*; Academic Press Inc.: New York, 1983; pp 140–165.

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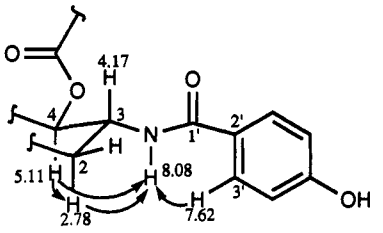
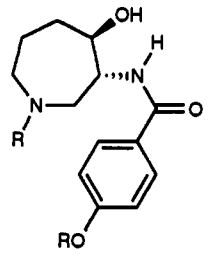


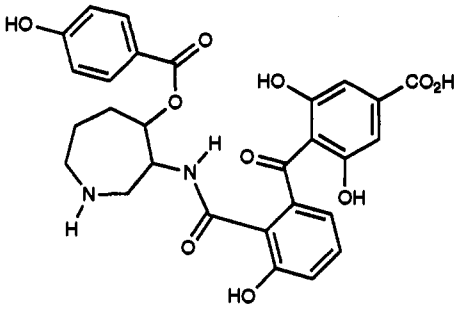
c

Hz) with H-3 and hence had to be located *trans* to H-3. To verify the proposed relative stereochemistry and to establish the absolute configuration of **1**, an X-ray analysis of **3**, a *p*-bromobenzoyl derivative of **2**, was undertaken. The results are presented in Figure 1, which shows the *anti* disposition of the substituents at C-3 and C-4 and the absolute configuration as 3*R*,4*R*.



2 R = H

3 R = *p*-bromobenzoyl



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It is of interest that ophiocordin (**4**),[5] $[\alpha]_D$ +70° (*c* 1, MeOH), an antifungal antibiotic from the fungus *Cordyceps ophioglossoides*, was reported to have a regioisomeric structure of balanol.

Balanol represents a novel class of protein kinase C inhibitors. IC_{50} values of 4–9 nM were observed in assays against human PKC enzymes α, β-I, β-II, γ, δ, ϵ, and η with the exception of ζ (150 nM). The inhibition profile is similar to those of staurosporine[6] and its congeners.[7]

Table I. ^1H NMR (600 MHz) and ^{13}C NMR (75.5 MHz) Spectral Data of Balanol in DMSO-d_6

position	^1H	^{13}C
2a	2.90 (dd; 14.6 3.8)	50.14
2b	2.78 (dd; 14.6, 7)	
3	4.17 (dddd; 8.7, 7.6, 7, 3.8)	55.36
4	5.11 (ddd; 8.1, 7.6, 3.8)	78.00
5a,5b	1.89 (m)	29.05
6a	1.74 (m)	24.77
6b	1.60 (m)	
7a	2.81 (ddd; 13, 6, 6)	48.22
7b	2.73 (ddd; 13, 8.1, 6)	
1′		165.87
2′		125.66
3′,7′	7.62 (d; 8.7)	129.44
4′,6′	6.74 (d; 8.7)	115.09
5′		160.43
1″		165.40
2″		134.77
3″,7″	6.66 (s)	107.99
4″,6″		159.83
5″		120.12
8″		201.44
9″		129.90
10″		153.43
11″	6.64 (brd; 7.6)	116.59
12″	7.07 (t; 7.6)	129.03
13″	6.96 (brd; 7.6)	118.70
14″		141.41
15″		171.78
CONH	8.08 (d; 8.7)	

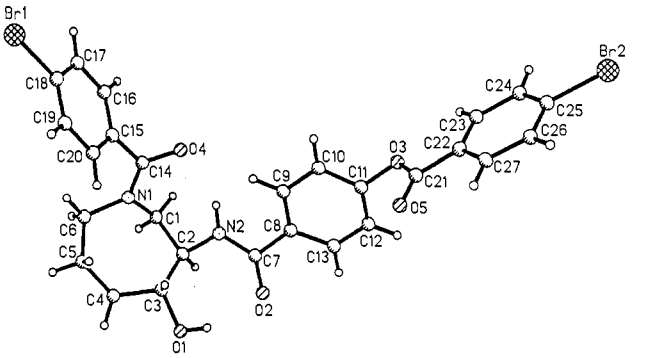

Figure 1. A computer-generated perspective drawing of the final X-ray model of **3**. The absolute configuration was determined from the anomalous scattering from the bromines.

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Supplementary Material Available: NMR data for compounds **1**, **2**, and **3** and listing of crystallographic data for compound **3** (6 pages). Ordering information is given on any current masthead page.

(5) (a) Kneifel, H.; Kõnig, W. A.; Loeffler, W.; Müller, R. *Arch. Microbiol.* **1977**, *113*, 121–130. (b) Kõnig, W. A.; Sinnwell, V.; Witt, S.; Kneifel, H. *Chem. Ber.* **1980**, *113*, 2221–2226.

(6) (a) Tamaoki, T.; Nomoto, H.; Takahashi, I.; Kato, Y.; Morimoto, M.; Tomita, F. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 397–402. (b) McGlynn, E.; Liebetanz, J.; Reutener, S.; Wood, J.; Lydon, N. B.; Hofstetter, H.; Vanek, M.; Meyer, T.; Fabbro, D. *J. Cell. Biochem.* **1992**, *49*, 239–250.

(7) (a) Takahashi, I.; Kobayashi, E.; Asano, K.; Yoshida, M.; Nakano, H. *J. Antibiot.* **1987**, *40*, 1782–1784. (b) Takahashi, I.; Kobayashi, E.; Asano, K.; Kawamoto, I.; Tamaoki, T.; Nakano, H. *J. Antibiot.* **1989**, *42*, 564–570. (c) Osada, H.; Koshino, H.; Kudo, T.; Onose, R.; Isono, K. *J. Antibiot.* **1992**, *45*, 189–194. (d) Koshino, H.; Osada, H.; Isono, K. *J. Antibiot.* **1992**, *45*, 195–198. (e) Kinnel, R. B.; Scheuer, P. J. *J. Org. Chem.* **1992**, *57*, 6327–6329.