APHE-1 AND APHE-2, TWO NEW ANTIMICROBIAL AND CYTOTOXIC ANTIBIOTICS

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE ELUCIDATION

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Two new pyrazolo-isoquinolinone antibiotics, APHE-1 and APHE-2, have been isolated from the culture filtrate and mycelia of *Streptoverticillium griseocarneum* NCIMB 40447. Molecular formulae were established as $C_{13}H_{12}N_2O$ for APHE-1 and $C_{14}H_{14}N_2O$ for APHE-2, by elemental analysis, NMR and mass spectra. 2D NMR techniques (^{1}H-^{1}H COSY-45 and ^{1}H-^{13}C correlated spectroscopy) have been applied to establish their structures.

In the preceding paper we have described the fermentation, isolation and biological activities of APHE-1 and APHE-2, two new antimicrobial and cytocidal antibiotics[1]. In this paper, we report the physico-chemical properties and structural elucidation of APHE-1 and APHE-2.

Physico-chemical Properties

The antibiotics APHE-1 and APHE-2 were isolated as yellowish powders. They were easily soluble in methanol, ethanol, acetone, ethyl acetate and chloroform, slightly soluble in benzene but insoluble in hexane, petroleum ether and water. Both compounds gave negative ninhydrin reaction. The molecular formulae were determined to be $C_{13}H_{12}N_2O$ for APHE-1 and $C_{14}H_{14}N_2O$ for APHE-2 by EI-MS and elemental analysis. Additional physico-chemical and chromatographic properties are given in Table 1.

Table 1. Physico-chemical properties of APHE-1 and APHE-2.

	APHE-1	APHE-2
Appearance	Yellow powder	Yellow powder
MP	157~159°C	130~132°C
Molecular formula	$C_{13}H_{12}N_2O$	$C_{14}H_{14}N_2O$
EI-MS (*m/z*)	213 $(M+1)^+$, 212 $(M)^+$, 197 $(M-15)^+$, 156, 144, 142, 128, 116, 89	227 $(M+1)^+$, 226 $(M)^+$, 197 $(M-29)^+$, 156, 144, 142, 128, 116, 89
Elemental analysis	$C_{13}H_{12}N_2O \cdot \frac{1}{2}H_2O$	$C_{14}H_{14}N_2O \cdot \frac{1}{2}H_2O$
Calcd:	C 70.59, H 5.88, N 12.67	C 71.49, H 6.38, N 11.91
Found:	C 70.33, H 5.56, N 12.32	C 71.80, H 6.54, N 12.02
UV λ_{max}^{MeOH} nm	265, 280 and 296	265, 280 and 296
IR ν_{max} (KBr) cm^{-1}	3134, 1640, 1579, 1451, 1376, 1254, 1121, 1001, 770, 743	3167, 1635, 1575, 1452, 1382, 1252, 1121, 1005, 777, 738
Rf value[a]	0.63	0.66

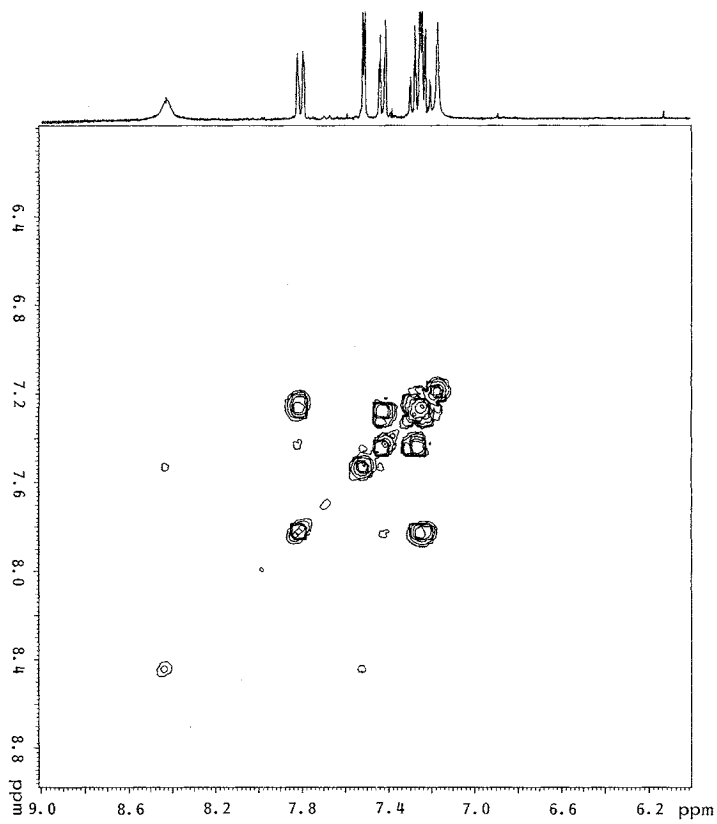
[a] Silica gel TLC (Merck); solvent: chloroform-methanol (97:3).

Structural Determination

The ion $m/z=197$ was present in both MS spectra (Table 1) and was orginated from M^+ peak (base peak) by the loss of a fragment of 15 (CH_3) and 29 (C_2H_5) mass units for APHE-1 and APHE-2, respectively. The fragmentation sequence from this ion led to the same essential fragment ions, suggesting the presence of the same or a similar ($C_{12}H_9N_2O$) structural unit. The UV spectra (Table 1) were identical for both compounds and exhibited characteristic absorption maxima at 265, 280 and 296 nm, indicating a similar aromatic chromophore. Their IR spectra (Table 1) showed an amide carbonyl absorption at 1640 and 1579 cm^{-1} for APHE-1 and 1635 and 1575 cm^{-1} for APHE-2 as well as a NH band.

The ^1H and ^{13}C NMR spectra in $CDCl_3$ solutions exhibited very simple signals in the upfield region which were attributed to an ethyl and a *n*-propyl group for APHE-1 and APHE-2, respectively, in agreement with MS spectra. The remaining signals of the two compounds, due to a highly unsaturated ($C_{11}H_7N_2O$) moiety, appeared at low field and showed similar chemical shifts and multiplicities. The wide singlet at *ca.* δ 8.5 in the ^1H NMR spectra (shifted at δ 10.63 for APHE-2 in acetone-d_6 solution), exchanged with D_2O and was attributed to NH proton. The signal at *ca.* δ 163 in the ^{13}C NMR spectra confirmed the amide carbonyl group. Between δ 7.1 ~ 7.9 there were six signals for methine groups which were confirmed by the corresponding carbon resonances in the ^{13}C NMR spectra. The spectra also exhibited four quaternary carbon signals in the region δ 105 ~ 148. These facts suggested the same

Fig. 1. ^1H-^1H COSY-45 spectrum of APHE-2 (299.949 MHz, $CDCl_3$).



features for this structural unit and agreed with an heteroaromatic system.

In order to propose a plausible structure for these compounds the ^{1}H and ^{13}C NMR spectra of APHE-2 were analyzed in depth by application of 2D NMR techniques[2,3] and double resonance (DR) experiments. In particular, COSY-45[4] and heteronuclear ^{1}H-^{13}C correlated spectroscopy (XHCORD)[5,6] were used.

The COSY cross-connectivity patterns (Fig. 1) revealed that the doublet at δ 7.53 only exhibited a large correlation with the NH signal, and no correlations were found for the slightly broadened singlet at δ 7.19. A weak cross-peak at δ 7.82/7.44 and two strong cross-peaks at δ 7.82/7.25 and 7.44/7.25 were observed. To strengthen these facts, DR experiments were performed. Thus, the saturation of the multiplet at δ 7.82 simplified the signal at δ 7.44 into a doublet of doublets with splitting of *ca.* 7.0 and 1.5 Hz. This experiment also showed a loss of a coupling of approximately 1.5 Hz at the signal centered at δ 7.29 which appeared as an apparent triplet with a splitting of *ca.* 7.0 Hz. Further interpretation of DR experiments, in which the signals at δ 7.44, 7.53 and 8.44 were saturated, confirmed the above considerations. The study of the ^{1}H and COSY-45 spectra and DR experiments in acetone-d_6 led to the same results.

The analysis of the respective signals allowed the establishment of the proton magnetic parameters of APHE-1 and APHE-2 collected in Table 2. On the basis of these statements, the four-spin ABMX system at δ 7.25, 7.29, 7.44 and 7.82 was attributed to a 1,2-disubstituted phenyl ring. The value of the coupling constants between the CH proton at 7.53 and the NH proton (2.6 Hz) was similar to those found in five-membered heteroaromatic systems[7,8] and is compatible with the presence of a substituted diazole. Moreover, both rings might be linked by the carbonyl amide and the isolated CH (δ 7.19) groups.

^{13}C chemical shifts are tabulated with signal multiplicity, obtained from DEPT experiments, in Table 2. Once the resonances of the respective protons were established, the analysis of the ^{1}H-^{13}C correlated spectrum of APHE-2 allowed the identification and assignment of the following signals: δ ($^{13}C/^{1}H$)

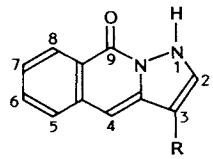
Table 2. ^{1}H and ^{13}C NMR data for APHE-1 and APHE-2 (299.949/75.429 MHz, $CDCl_3$).

^{1}H δ (ppm)[a]			J_{HH} (Hz)[b]			^{13}C δ (ppm)[c]		
Assignment	APHE-1	APHE-2	Assignment	APHE-1	APHE-2	Assignment	APHE-1	APHE-2
1-H, br s	8.53	8.44	1-H~2-H	2.6	2.6	C-2, d	121.53	121.46
2-H, d	7.53	7.53	5-H~6-H	7.4	7.6	C-3, s	105.82	106.00
4-H, s	7.17	7.19	5-H~7-H	1.4	1.4	C-3a, s	147.21	147.00
5-H, m	7.44	7.44	5-H~8-H	0.8	0.8	C-4, d	119.36[d]	119.72[d]
6-H, m	7.31	7.29	6-H~7-H	7.1	7.1	C-4a, s	136.14	136.18
7-H, m	7.23	7.25	6-H~8-H	1.5	1.6	C-5, d	111.46	111.44
8-H, m	7.83	7.82	7-H~8-H	7.3	7.4	C-6, d	122.94	122.90
1'-H	2.90, q	2.87, t	1'-H~2'-H	7.5	7.4	C-7, d	120.79	120.75
2'-H	1.42, t	1.90, m	2'-H~3'-H	7.5	7.4	C-8, d	119.87[d]	119.92[d]
3'-H, t	—	1.06				C-8a, s	124.00	124.06
						C-9, s	163.69	163.40
						C-1', t	21.62	30.70
						C-2'	11.22, q	20.58, t
						C-3', q	—	13.70

[a] Error $\pm$0.01 ppm.
[b] Error $\pm$0.1 Hz.
[c] Error $\pm$0.02 ppm. Signal multiplicity was deduced from the DEPT experiments.
[d] Values may be interchanged.

13.70/1.06 (CH_3), 20.58/1.90 (CH_2), 30.70/2.87 (CH_2), 120.75/7.25; 122.90/7.29; 111.44/7.44; 119.92/7.82 (CH, 1,2-disubstituted phenyl ring), 119.72/7.19 (isolated CH) and 121.46/7.53 (*CH*-NH, diazole system). The signals at 119.72 and 119.92 could not be distinguished and the correlation with the proton signal at δ 7.19 was very weak.

Fig. 2. Structures of APHE-1 and APHE-2.



R: $-\overset{1'}{C}H_2-\overset{2'}{C}H_3$(APHE-1)

$-\overset{1'}{C}H_2-\overset{2'}{C}H_2-\overset{3'}{C}H_3$ (APHE-2)

Discussion

APHE-1 and APHE-2 are novel antibiotics produced by *Streptoverticillium griseocarneum* NCIMB 40447. The spectroscopic studies have established that these compounds are condensed heterotricycles which only differ in the nature of an aliphatic chain; an ethyl and a *n*-propyl group for APHE-1 and APHE-2, respectively. Taking into account the spectroscopic data as well as substituent steric and electronic effects on proton[7,8] and ^{13}C chemical shifts[8,9] and the magnitude of the ^1H-^1H coupling constants[7,8], a b-fused 1(2*H*)-isoquinolinone system is proposed as the most probable structure. These antibiotics are considered as 3-substituted 1*H*-pyrazolo[2,3-*b*]isoquinolin-9-ones (Fig. 2). This structure is favored over the alternative 2-substituted or imidazoisoquinolinone structures on the basis of spectral data. The assignment of the respective proton and carbon signals for the proposed structure are shown in Table 2.

Experimental

The melting points were uncorrected. The IR spectra were recorded on KBr pellets on a Perkin-Elmer 883 spectrophotometer. UV spectra (λ_{max} in nm) were recorded in MeOH solutions on a Beckman DU-50 spectrometer. The mass spectra were carried out in a Hewlett-Packard 5987A spectrophotometer by EI. The elemental analysis were performed on a Perkin-Elmer 240B elemental analyzer. The ^1H, ^{13}C and 2D NMR spectra as well as double resonance (DR) and DEPT experiments were recorded on a Varian UNITY-300-FT spectrometer (299.949 MHz for ^1H and 75.429 MHz for ^{13}C), using solutions *ca.* 0.02 M in $CDCl_3$ and acetone-d_6 with TMS as the internal reference, at 293°K.

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