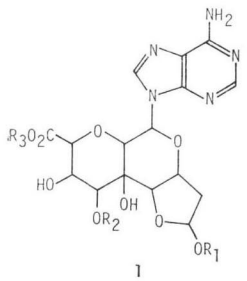
THE REVISED STRUCTURE OF HERBICIDINS

Sir:

In our previous papers, we reported the isolation, biological activities and general characterization of herbicidins A, B, E, F and G produced by *Streptomyces saganonensis*.[1~4] Herbicidins are a family of unique antibiotics possessing herbicidal activity. Tentative structures for the herbicidins had been proposed as **1** on the basis of chemical degradation studies and physico-chemical data.[5] During the course of our efforts directed toward the chemical modification of herbicidins, we found structure **1** to be incorrect. In this paper, we report the revised structures **2a**, **b**, **c**, **d**, **e**, for the herbicidins.

Herbicidin A, **2a**; $C_{23}H_{29}O_{11}N_5$, mp 133°C (dec.), $[\alpha]_D^{20}+61.7°$ (*c* 1, MeOH), molecular weight 551 (FD mass spectrum) and herbicidin B, **2b**; $C_{18}H_{23}O_9N_5$, mp 155°C (dec.), $[\alpha]_D^{20}+63°$ (*c* 1, MeOH), molecular weight 453 (FD mass spec-



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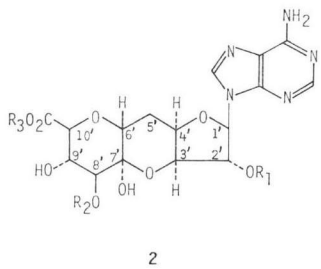

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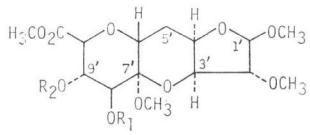
		R_1	R_2	R_3
a	Herbicidin A	CH_3	$CO(CH_2OH)C\overset{E}{=}CHCH_3$	CH_3
b	Herbicidin B	CH_3	H	CH_3
c	Herbicidin E	CH_3	$COCH(CH_3)_2$	CH_3
d	Herbicidin F	CH_3	$CO(CH_3)C\overset{E}{=}CHCH_3$	CH_3
e	Herbicidin G	H	$CO(CH_3)C\overset{E}{=}CHCH_3$	H

Table 1. Chemical shift (δ).

	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′	H-8′	H-9′	H-10′	Solvent
2a	6.12	4.42	4.53	4.09	~2.26	4.59	5.09	4.38	4.50	CD_3OD
2b	6.16	3.99	4.44	4.42	~2.23	4.67	3.79	4.35	4.38	CD_3OD
3a	4.87	3.75	3.99	4.47	2.13 2.18	4.61	3.96	4.35	4.39	$CDCl_3$
3b	4.74	3.54	3.89	4.34	2.27 2.30	4.71	5.63	5.79	4.64	$CDCl_3$
4	6.25	4.14	5.28	4.54	~2.40	5.20				$CDCl_3$
5	4.87	3.83	5.18	4.29	2.10 2.40	5.51				C_6D_6
6	4.60	3.40	3.70	4.10	~2.18	4.05	5.35	5.74		CCl_4
7a	5.96	4.07	4.12	4.13	~2.10	5.22	4.21	3.59	4.21	DMSO-d_6
7b	6.27	4.18	5.31	4.67	~2.30	5.39	5.78	5.51	4.43	$CDCl_3$

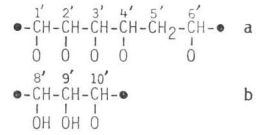
Table 2. Approximate *J* values (Hz).

	1′,2′	2′,3′	3′,4′	5′a,5′e	4′,5′a	4′,5′e	6′,5′a	6′,5′e	8′,9′	9′,10′
2a	~1.5	≥0	2~2.5		3	3	8~9	8~9	3.5	1.5
2b	~1.2		2.5		3	3	8~9	8~9	3	1.5
3a	≥0	≥0	3	14	3	3	10.5	7	3	1.5
3b	≥0	≥0	3	13	3	3	11.5	5.5	3	1.2
4	~1.0	~1.7	5.4	14	10	3	10.8	3.2		
5	1.0	1.8	5	13	9	4	10.8	3.2		
6	≥0	≥0	3	14	3	3	11	7	5.8	
7a	≥1.0								8	5
7b	≥1.0	≥0	3.5		8	5.5	9	4	8	5



3a $R_1 = R_2 = H$

3b $R_1 = R_2 = COC_6H_4Br(p)$

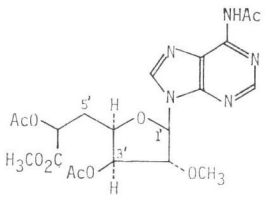


trum) were obtained as a basic white amorphous powder and as basic white crystals, respectively. The presence of a N-9 substituted adenine moiety in herbicidins was detected by the following evidence; a) Acid hydrolysis of herbicidins afforded adenine. b) UV absorption at λ_{max} 259 nm. c) ^1H and ^{13}C NMR spectra (for example, signals at 8.17 and 8.47 ppm for ^1H NMR and signals at 119.9, 141.6, 153.5 and 156.1 ppm for ^{13}C NMR of **2b**). The ^1H NMR and ^{13}C NMR spectra of herbicidins **2a**, **b**, **c**, **d** indicated the presence of the same skeletal structure with different acyl components. On mild base hydrolysis (0.1 N KOH, 50°C, 2 hours), **2a**, **2c** and **2d** afforded a (*E*)-2-hydroxymethyl-2-butenoic acid,[6] *iso*-propionic acid and tigric acid, respectively. The ^{13}C NMR spectrum of **2b** indicated the existence of a methoxy methyl group (58.9 ppm), methyl ester (53.7 and 172.3 ppm), methylene (25.6 ppm), one quaternary carbon (93.9 ppm) and eight carbons attached to oxygen (70.5, 72.4, 73.6, 77.4, 79.5, 88.9 and 90.7 ppm). The ^1H NMR spectra coupled with decoupling experiments (Tables 1 and 2) on the methanolysis product (MeOH/amberlyst 15/90°C) of herbicidin B, **3a**; oil, $C_{15}H_{24}O_4$, M^+ 364 and its di-*p*-bromobenzoyl ester, **3b**; $C_{29}H_{30}O_{12}Br_2$, mp 183~184°C, revealed the following partial structures **a** and **b** in **3a** and **3b**, respectively.

Sodium periodate oxidation of **2b** in aqueous acetic acid followed by esterification and acetylation afforded **4**; oil, $C_{20}H_{25}O_9N_5$, M^+ 479 (FD mass spectrum). A fragment peak at 175 corresponded to $C_7H_7ON_5$ in the FD mass spectrum and the chemical shifts δ at 8.25 (H), 8.7 (H), 2.6 (N_9–Ac), 9.2 (N*H*–Ac) were assigned to the N_9-acetyl adenine moiety. From the signals in the ^1H NMR spectrum δ at 2.03 (Ac), 2.13 (Ac) and 3.68 (CO_2CH_3), and an unsaturation number of 11 (including adenine), compound **4** required a monocyclic ether structure connected to N_9-acetyl adenine. The position at which adenine is attached to this monocyclic ether structure **4** was confirmed as follows: Methanolysis (5% HCl - MeOH) of **4** gave **5**; oil, $C_{14}H_{22}O_9$, M^+ 334. The ^{13}C NMR spectrum of **5** showed a signal at 107.5 ppm assigned to the C-1′ carbon of furanosyl moiety. The analysis of the ^1H NMR spectra (Tables 1, 2) of **4** and **5** indicated that the partial structure **a** contains an adenine furanosyl structure and the methoxy group is substituted to the C-2′ position of the furanosyl part.

The structural information about the partial structure **b** were provided as follows. The hydrogenation, methanolysis and dehydrolysis of herbicidin A, **2a** gave an anhydro derivative **6**; oil, $C_{20}H_{30}O_{10}$, M^+ 430. The UV spectrum (λ_{max}^{EtOH} 238 nm, ε 7,070) of **6** revealed an unsaturated glucuronic acid moiety.[7] The IR spectrum of **6** showed absorption bands due to an unsaturated ester and an enol double bond at 1728 and 1650 cm^{-1}. In the ^{13}C NMR spectrum, the signals at 107.5 and 145.7 were assigned to C-9′ carbon and C-10′ carbon, respectively. The ^1H NMR spectral analysis of **6** (Tables 1, 2) revealed that the partial structure **b** is a glucuronic sugar type structure in which 8′-hydroxyl group is acetylated.

The existence of an O–C–O quaternary carbon in **2b** was shown from the ^{13}C NMR spectrum (93.1 ppm) and the appearance of an extra methyl ether signal δ at 3.14 in **3a**, suggested the presence of a hemiketal function to which the adenofuranosyl part and the glucuronic part might be con-



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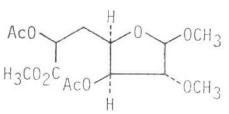

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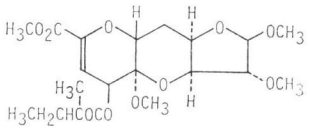

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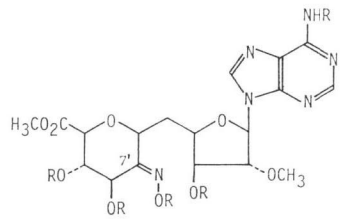
nected. Oxime formation at the hemiketal part provided a complete structural information. Treatment of herbicidin B, **2b**; with hydroxylamine in methanol containing sodium acetate gave an oxime derivative **7a**; $C_{18}H_{24}O_9N_6$, mp 191~192°C, which can easily be reformed to herbicidin B, **2b** by a mild acid treatment. A comparison of the ^{13}C NMR spectra of **2b** and **7a**, indicated that an O–C–O quaternary carbon signal, δ, at 93.9 in **2b** shifted to 155.8 in **7a**. The ^1H NMR spectral analysis (Tables 1 and 2) of an acetate **7b** ($C_{30}H_{36}O_{15}N_6$, m.p. 139~141°C) also indicated that the oxime was formed at the C-7′ position.

Based on all the above evidence, the revised structures **2a**, **b**, **c**, **d**, **e**, were shown to be the only possible ones for the herbicidins. Because of this unusual tricyclic ring structure, the relative configuration of the herbicidins could not be proposed on the basis of the NMR coupling constants. However the following nuclear Overhauser effects indicated the vicinal *trans*, *trans* relation of 8′-H, 9′-H, 10′-H and the *trans* ring junction at C-6′, C-7′. Irradiation of 7′-methoxy methyl signal in **3b** affects 8′-H (*ca.* 14% increase in signal area), whereas the same irradiation



7a R = H
7b R = Ac

Fig. 1.

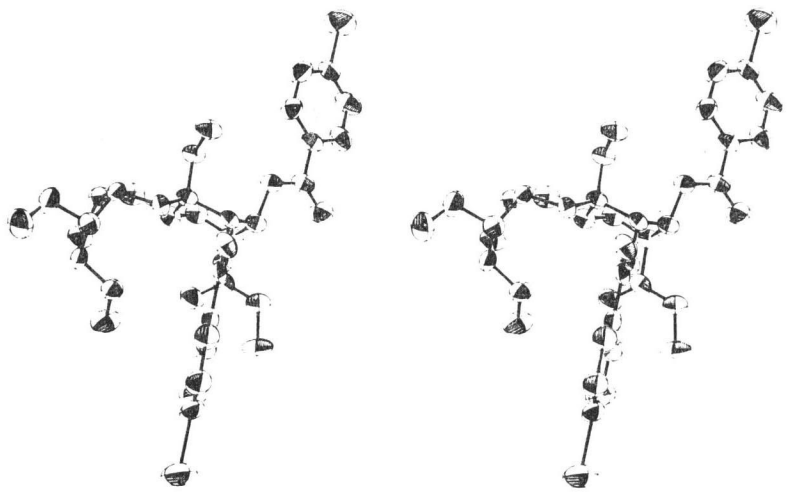
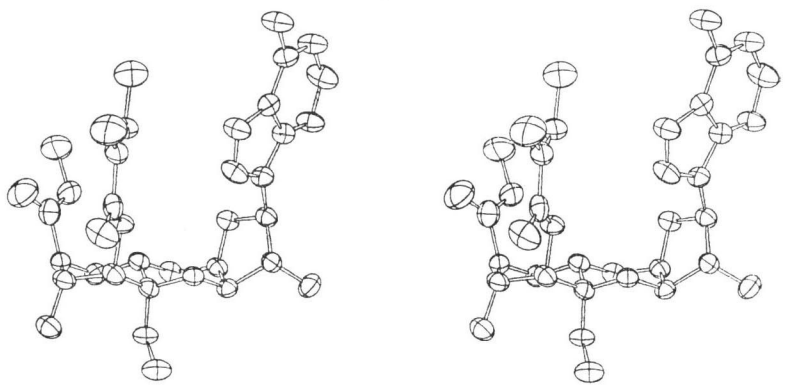
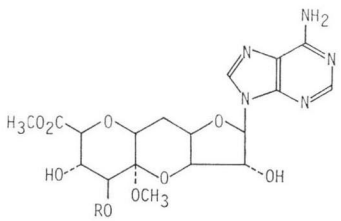


Fig. 2.



causes no effect on 6′-H. On the other hand, irradiation of 9′-H in **3b** produces a greater effect on 10′-H (*ca.* 18% increase in signal area). The *cis* relation of adenine and 2′-H was also suggested by a nuclear Overhauser effect. Irradiation of 2′-H in herbicidin A methyl ether affects 8-H of adenine (*ca.* 8% increase in signal area).

The complete structure was elucidated by means of X-ray crystallography of **3b** and of **8** ($C_{23}H_{29}O_{10}N_5 \cdot CHCl_3$), obtained by methylation of herbicidin G, **2e**, with diazomethane. **3b** is orthorhombic, P 2_12_12 with a=27.759(5), b=13.451(1) and c=8.149(1) Å. 2418 unique intensity date for $2\theta<128°$ were collected on a Rigaku four-circle diffractometer with graphite-monochromated CuKα radiation. The structure has been determined by Patterson and Fourier techniques and refined by block-diagonal least-squares methods to an R-factor of 0.082. The absolute configuration of **3b** has been established from eleven enantiomer-sensitive Bijvoet reflection pairs. Compound **8** is orthorhombic, P $2_12_12_1$ with a=10.800 (2), b=12.168 (2) and c=22.142 (2) Å. Intensities were measured out $2\theta=128°$ with monochromatic CuKα radiation. After Lorentz and polarization correction, 2220 reflections with $F \geq 3\sigma(F)$ were used in the calculations. The structure was solved by direct methods and refined by least-squares methods. The final R-value was 0.055. The molecular skeletons of **3b** and **8** thus obtained are shown in Fig. 1 and Fig. 2, respectively.



8 R = $COCH_3C{=}CHCH_3$

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