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BOTRYODIPLODIN: A MYCOTOXIN FROM *PENICILLIUM ROQUEFORTI*

REACTION WITH AMINO-PYRIMIDINES, AMINO-PURINES AND 2'-DEOXYNUCLEOSIDES

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Abstract—Botryodiplodin is a mycotoxin isolated from a strain of *Penicillium roqueforti*. This toxin exhibits genotoxic properties, it inhibits cell metabolism at the level of replication, transcription and traduction; it induces DNA-protein cross-links. The chemical reaction with amino-pyrimidines and amino-purine has led to 2-aminooxolane compounds. The structure and the stereochemistry of these adducts have been studied. Likewise the reaction with various 2'-deoxynucleosides has led to similar compounds.

Toxic metabolites from *Penicillium roqueforti*, a fungal species used in the ripening of various blue veined cheeses, have been reported by various authors. For example Wei[1] isolated and identified the PR toxin from the culture media of a strain of this fungal species. From various other strains, we have isolated closely related metabolites such as the eremofortins A, B, C, D and E.[2] Recently we have turned our interest towards non PR toxin producing strains of this species, and we isolated a new toxin: the botryodiplodin[3] (1).

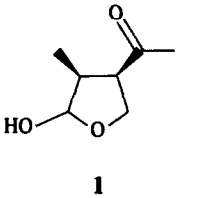
The mycotoxin botryodiplodin was first isolated from culture of *Botryodiplodia theobromae* Pat, a widespread fungus in tropical countries.[4] This fungal species produces considerable damage in many tropical plants and is in particular a cause of extensive wastage in banana fruit cultivation.[5]

Botryodiplodin has been shown to inhibit the growth of some Gram-positive and Gram-negative bacteria.[4] Moulé recently investigated the various biological properties of this mycotoxin. The LD 50 is 40–50 mg/kg for adult mice. The toxin inhibits cell multiplication in growing cultures. It perturbed the metabolism of cultured mammalian cells at the level of replication, transcription and translation.[6] The toxin exhibits a mutagenic activity in the *Salmonella typhymurium* test.[7] It was also found that botryodiplodin induces sister-chromatid exchanges in cultured cells.[8]

Furthermore the toxin interacted with DNA in eucaryotic cells, this interaction consisted in the formation of cross-links between DNA and protein.[9] When considered together these data clearly establish the genotoxicity of botryodiplodin. All the genotoxic properties are obtained without metabolic activation, that means that the active molecule is really botryodiplodin. In fact we have shown that the single methylation of the hemiacetalic group completely suppresses all the biological properties, in particular the capacity to induce DNA-protein cross-links.[8,9] Thus the hemiacetalic function was essential for the biological activity.

In order to investigate the genotoxic properties of this toxin we chose to examine the chemical reactivity of botryodiplodin towards nucleic acids. This paper reports a first study of the reaction between botryodiplodin and various models of nucleic acid bases. The models chosen are amino-pyrimidines (2-amino-pyrimidines, 2,6-dimethyl-4-amino-pyrimidines, 1-propyl-cytosine), amino-purine (9-propyl-adenine, 9-propyl-guanine) and finally the various 2'-deoxynucleosides.

We first examined the structure of the O-methyl derivative of botryodiplodin (3) (used in the biological



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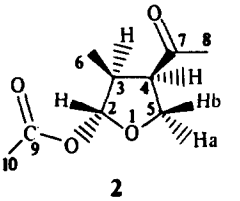

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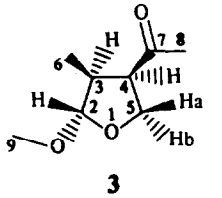


3

tests) by comparison with the previously reported structure of the botryodiplodin acetate (2).[3] This study happened to be very useful in the chemical

determination of the adducts between botryodiplodin and pyrimidic and puric compounds.

Structures of botryodiplodin acetate (2) *and O-methyl botryodiplodin* (3)

Acetylation of botryodiplodin yield a unique compound (2). The relative stereochemistry has been determined[10] and confirmed by X-ray analysis.[3] This molecule was characterized by a *cis* relationship between protons 3 and 4, and a *trans* relationship between protons 2 and 3.

The PMR spectrum (350 MHz) showed the expected chemical shifts (Table 1), as well as the characteristic spin–spin splitting patterns (Table 2). The singlet exhibited by proton H-2 (δ 5.98) was in agreement with a *trans* stereochemistry between H-2 and H-3. The two H-5 protons showed well separated resonances at 350 MHz. H_a-5 experiencing the shielding of the *cis* ketone group showed resonance at a higher chemical shift. H_b-5 (δ 4.35) showed a coupling constant ($J_{H_b\text{-}5,H\text{-}4}$ = 9 Hz) consistent with the *cis* configuration of these protons.

The structure of O-methyl botryodiplodin (3) obtained by methylation of botryodiplodin with iodomethane and silver oxide,[11] was easily deduced from the ^{1}H-NMR and ^{13}C-NMR spectra (Tables 1, 2 and 3). The stereochemistry of 3 is discussed later.

Structures of the various adducts

The reaction of botryodiplodin with the various amino compounds led to the isolation of 2-aminooxolane derivatives (**4**, **5**, **6**, **7a** and **7b**).

Structures of **4**

The structure was assigned on the basis of the following data. The PMR spectrum (350 MHz, Fig. 1) showed the presence of the oxolane skeleton of botryodiplodin, characterized by the methyl doublet (δ 1.22) and the methyl ketone singlet (δ 2.24). The protons H-3, H-4, H-5 showed resonances at the expected values as already observed in (2) (Table 1), and the attribution have been confirmed by double resonance experiments. The ^{13}C chemical shifts and the multiplicity observed in the off-resonance spectrum were in concordance with the oxolane ring. The aromatic moiety of the molecule was deduced from the presence of the aromatic protons which exhibited resonances at the expected values[12] (Table 1). The substitution at C-2 of the oxolane ring by a nitrogen atom was shown by the chemical shift of H-2 in the PMR spectrum (δ 5.59), confirmed by the chemical shift and the multiplicity of C-2 in the CMR spectrum (δ 89.1) as expected for such substituted carbon atoms.[13] H-2 exhibited two coupling constants due to H-3 (J = 7.3 Hz) and H-9 (J = 9 Hz).

The high resolution mass spectrum of **4** gave a molecular ion at *m/e* 221.1163 confirming thus the elemental composition $C_{11}H_{15}N_3O_2$ (calc 221.1164).

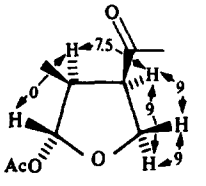
Structures of **5**, **6**, **7a** *and* **7b**

These structures were determined in the same way. The NMR spectral data are shown in Tables 1, 2 and 3. Double resonance experiments were measured on each compound. The observed resonances for the various aromatic heterocyclic nucleus were in agreement with the available literature data for ^{1}H and ^{13}C.[12,14]

Stereochemistry

The stereochemistry of **2** determined by X-ray diffraction allowed us to reach the stereochemistry of the adducts, and of the O-methyl derivative (3). The interpretation of spin–spin splitting constants between H-2, H-3, H-4 and Ha,6-5 was the key to this determination.

The careful examination of Tables 1 and 3 showed that the various compounds could be gathered into 2 main categories: **4**, **5**, **6** and **7a** on one hand, **2**, **3** and **7b** on the other hand.



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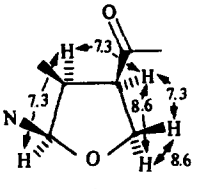

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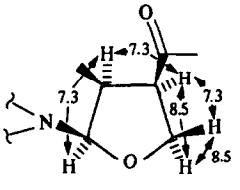
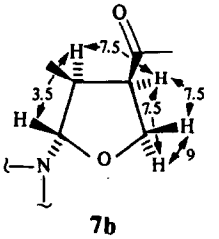


7a



7b

Table 1 shows that the chemical shift of H-4 was around 3 ppm for the first category and 3.5 for the second. In the same way, the ^{13}C chemical shifts (Table 3) of C-4 and C-6 underlined the same categories. C-4 δ

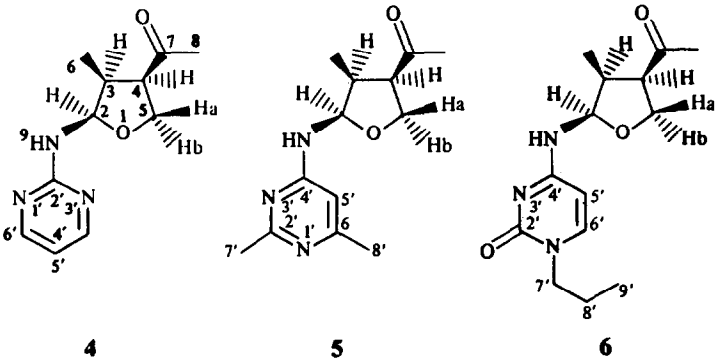


Table 1. Proton NMR chemical shifts (350 MHz) observed in $CDCl_3$ of botryodiplodin acetate **2**, O-methyl botryodplodin **3** and the various adducts: **4**, **5**, **6**, **7a** and **7b**

Compound	H-2	H-3	H-4	H_a-5	H_b-5	H-6	H-8	H-9	H-2′	H-4′	H-5′	H-6′	H-7′	H-8′	H-9′	H-10′	H-11′	H-12′
2	5.98	2.69	3.58	4.09	4.35	0.92	2.06											
3	4.66	2.58	3.57	3.93	4.29	0.86	2.19	3.33										
7b	5.95	2.68	3.55	4.10	4.26	1.12	2.24	6.36	8.46					7.80		4.19	1.93	0.96
4	5.59	2.44	3.01	3.98	4.21	1.22	2.24	6.03		8.36	6.66	8.36						
5	5.36	2.40	3.01	3.94	4.22	1.21	2.24	5.90			6.21		2.50	2.33				
6	5.62	2.46	2.99	3.95	4.18	1.19	2.25	5.89			6.26	7.22	3.75	1.76	0.92			
7a	5.95	2.54	3.03	4.00	4.24	1.23	2.29	6.86	8.44					7.81		4.14	1.90	0.93

Table 2. Spin–spin coupling constants observed at 350 MHz of the various compounds

Compound	$J^{*}_{H_2-NH}$	$J_{H_2-H_3}$	$J_{H_3-H_4}$	$J_{H_4-H_{a5}}$	$J_{H_4-H_{b5}}$	$J_{H_{a5}-H_{b5}}$	$J_{H_5-H_6}$
2		0	7.5 Hz	9.0 Hz	9.0 Hz	9.0 Hz	7.5 Hz
3		0	7.3 Hz	8.6 Hz	8.6 Hz	8.6 Hz	7.3 Hz
7b	8.0 Hz	3.5 Hz	7.5 Hz	7.5 Hz	7.5 Hz	9.0 Hz	7.5 Hz
4	9.0 Hz	7.3 Hz	7.3 Hz	7.3 Hz	8.6 Hz	8.6 Hz	7.3 Hz
5	9.0 Hz	7.3 Hz	7.3 Hz	7.3 Hz	8.5 Hz	8.5 Hz	7.3 Hz
6	13.0 Hz	7.0 Hz	†	5.6 Hz	8.6 Hz	8.6 Hz	7.0 Hz
7a	9.0 Hz	7.3 Hz	7.3 Hz	7.3 Hz	8.5 Hz	8.5 Hz	7.3 Hz

* Observed at 60 MHz.
† Wide signal $L_{1/2} = 35$ Hz.

Table 3. Carbon-13 NMR chemical shifts (15.1 MHz, $CDCl_3$)

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-2′	C-3′	C-4′	C-5′	C-6′	C-7′	C-8′	C-9′	C-10′	C-11′	C-12′
2	104.2	41.7	52.8	67.7	12.2	205.4	30.3	170.3	21.5											
3	110.7	42.0	53.3	66.3	12.5	204.3	29.5	54.0												
7b	88.6	42.4	54.0	67.0	12.9	206.5	30.5			152.9		149.0	119.8	153.8		140.4		45.5	23.4	11.1
4	89.1	41.6	59.0	67.6	17.0	207.4	29.8			161.7		158.1	112.3	158.1						
5	88.9	42.1	58.9	67.9	17.6	207.7	29.9			167.3		165.8	100.3	162.1	25.9	24.0				
6	87.7	41.8	58.6	68.0	17.6	206.7	29.9			209.2		164.0	93.6	156.4	51.9	22.3	11.0			
7a	88.4	42.2	59.0	68.0	17.2	207.7	29.9			152.8		149.2	120.2	154.2		140.7		45.4	24.3	11.1

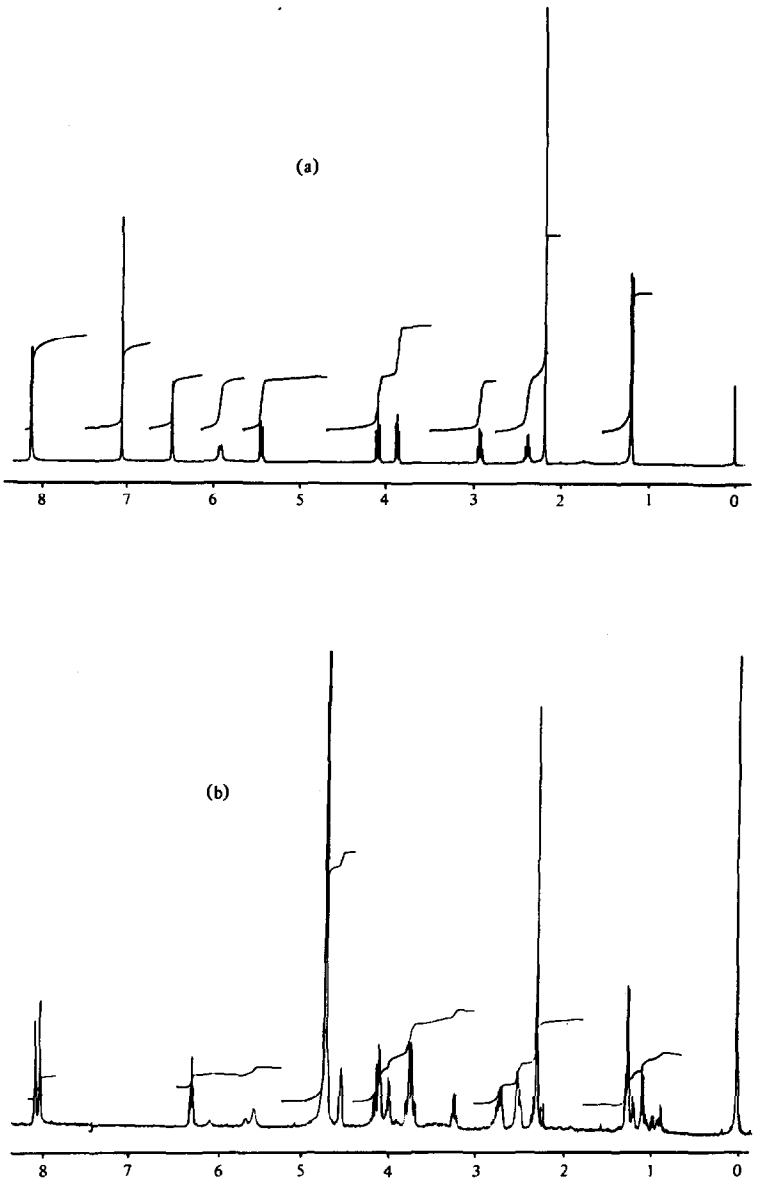


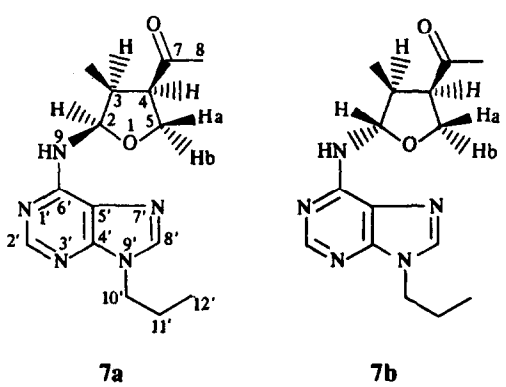
Fig. 1. ^{1}H-NMR spectra of **4** ($CDCl_3$) (a) and **8** (D_2O) (b).

$\simeq$ 59 (**4, 5, 6, 7a**) and $\delta \simeq$ 54 (**2, 3, 7b**); C-6 $\delta \simeq$ 17.5 (**4, 5, 6, 7a**) and $\delta \simeq$ 12.5 (**2, 3, 7b**).

Stereochemistry of **7a** *and* **7b**

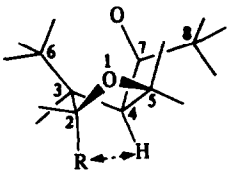
Compounds **7a** and **7b**, belonging to these two main categories, are epimeric at C-2. **7a** is the *cis* compound, **7b** the *trans*. This conclusion was deduced from the following observations.

As shown by Scheme 1 the spin–spin coupling constants of **7a** and **7b** had close values for H-3, H-4, H-5 spin systems. The main difference was due to H-2, H-3 coupling. The coupling constants for H-3, H-4, H-5 were closely related to the values observed for **2**, indicating the same stereochemistry for protons H-3, H-4, H-5 in compounds **7a**, **7b** and **2** (Scheme 1). The highest value for H-2, H-3 coupling constants (7.3 Hz, **7a**) indicated a *cis* relationship for these two protons.



7a **7b**

Scheme 1. Spin–spin coupling constant observed for compounds **2**, **4**, **7a** and **7b**.



Scheme 2. Dreiding model of **7b** showing the γ effect.

This configuration exhibits a dihedral angle close to 0° for the protons H-2, H-3 as shown by Dreiding models. On the other hand, the *trans* configuration showed a dihedral angle consistent with the lowest coupling constant observed in **7b** (3.5 Hz).

The previously mentioned differences in proton and carbon chemical shifts for **7a** and **7b** (Tables 1 and 3) can be explained by the proposed stereochemistry. The C-4 and C-6 carbon atoms of **7b** were more shielded than those of **7a**. Compound **7b** exhibits a 1,3 *cis* interaction between the substituent in position 2 and the H-4 proton (Scheme 2), inducing a γ effect on C-4.

The opposite configuration (**7a**) suppresses this interaction but induces a 1,2 vicinal steric effect between C-2 substituent and the methyl group (C-6). It has been shown in cyclopentane derivatives and tetrahydrofuranic compounds that this kind of interaction led to a deshielding effect for the interacting carbons[15] (this effect explains the observed shielding for C-6 in compound **7b**). The downfield shift of H-4 proton for (**7b**) was the result of the 1,3 *cis* interaction previously mentioned.[15]

Stereochemistry of **4**, **5** *and* **6**

Compounds **4**, **5** and **6** exhibited the same stereochemistry as **7a**. This was easily shown by the same arguments as those developed in the preceding case: a spin–spin coupling system for H-3, H-4, H-5 closely related to the system observed in **2**; a H-2, H-3 coupling constant of 7.5 Hz for the various compounds; a downfield shift for C-4 and C-6 carbon atoms.

Stereochemistry of **3**

It could easily be deduced from the various data that the stereochemistry of **3** is the same as **2**. In particular, we observed a zero spin–spin constant for H-2, H-3, therefore indicating a *trans* relationship for these two protons.

Attempts to confirm this stereochemistry by NOE measurements between H-2 and H-3 were unsuccessful. The nuclear Overhauser enhancements were weak and showed low reproducibility.

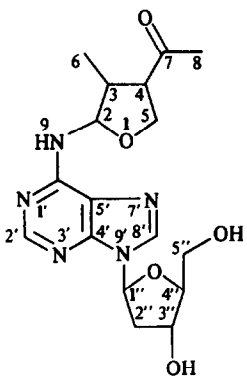
Structure of the adducts compounds with 2'-deoxynucleosides

The reaction between botryodiplodin and 2'-deoxyadenosine, 2'-deoxycytidine uniquely gave the expected 2-amino-oxolane derivatives with an overall yield of 5%. No reaction was observed with 2'-deoxyguanosine and thymidine.

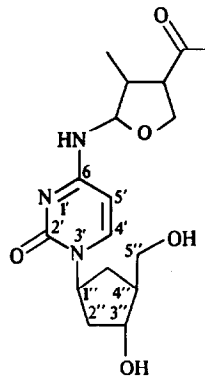
The structures **8** and **9** were assigned on the basis of the various spectral data by comparison with **7a**, **7b** and **6**. The complex 350 MHz ^{1}H-NMR spectra of **8** (Fig. 1) showed broad analogies with **7a** and **7b**. The oxolane skeleton of botryodiplodin was easily detected in the spectra. The methyl group and the methylketone group

Table 4. Proton NMR chemical shifts of adducts **8** and **9** (350 MHz, D_2O)

Compound	H-2	H-3	H-4	H_a-5	H_b-5	H-6	H-8	H'-2	H'-5	H'-6	H'-8	H''-1	H''_a-2	H''_b-2	H''-3	H''-4	H''-5
8	5.71	2.59	3.33	4.08	4.26	1.28	2.36	8.31			8.26	6.44	2.59	2.79	4.66	4.26	3.84
9	5.45	2.45	3.37	4.07	4.18	1.25	2.31		6.15	7.90		6.26	2.45	2.45	4.44	4.08	3.82



8



9

gave sharp resonances respectively at δ 1.28 (d, J = 7 Hz) and 2.36 (s). The H-3, H-4, H-5 spin system was also detected at the expected values (Table 4) and confirmed by double resonance experiments. The proton H-2 showed the characteristic resonance signal already observed in **7a**. Figure 1 clearly indicates that compound **8** was isolated with non-separable isomers as shown by the various H-6 systems detected at higher field. Moreover the puric protons and 2'-deoxyribose protons gave signals in concordance with the literature values.[13] The FAB mass spectrum showed peaks at *m/e* 400 $(M+Na)^+$ and *m/e* 375 $(M+H^+)$ in agreement with the structure. The structure of compound **9** was determined in the same way. ^{1}H-NMR data are reported in Table 1.

DISCUSSION

All the amino compounds used in this study reacted to yield a unique type of compound with botryodiplodin, i.e. 2-amino-oxolane derivatives. Clearly the hemiacetalic function is the unique functional group of the toxin implicated in such reactions. A reaction mechanism can be proposed for the formation of these compounds (Fig. 2). It involves the aldehydic group formed from the hemiacetal.[3] The imine thus formed undergoes an intramolecular cyclisation with the C-5 hydroxyl group. The reactivity exhibited by the botryodiplodin is weak. The optimum yield observed in the reaction with stoechiometric quantities of reactants is 20% (**7a**, **7b**). It must be noted that 1-propylguanine as well as 2'-deoxyguanosine do not lead to additive products with botryodiplodin. Such behaviour has already been observed with 2-chloroacetaldehyde on alkylated nucleic bases.[16]

To evaluate the biological implications of such reactions it should be necessary to examine the reactivity of botryodiplodin on DNA *in vitro* and *in vivo*, but the results presented in this work already led to two main observations. First, the reaction of the aldehyde group of the toxin with the amines does not lead to an imine as expected. The amino-oxolane obtained should under physiological condition of pH, exhibit a better stability than an imine, particularly towards hydrolysis reactions. Thus such compounds formed on DNA should have a sufficient life-time to allow the expression of the biological properties (inhibition of replication, transcription, traduction, and mutagenicity). Second, the mechanism of formation of the 2-amino-oxolane implies the intermediary formation of an imine. This imine instead of undergoing the internal cyclisation could react with another amine group (from proteins) and then lead to a bridge between the two amines. The formation of methylene bridges by such a mechanism is a well-known property of the common aldehyde formaldehyde.[17]

Though the results obtained here are compatible with the known biological properties of botryodiplodin but need to be tested on DNA by *in vitro* and *in vivo* studies.

EXPERIMENTAL

^{1}H-NMR spectra were recorded on a Cameca 350 (350 MHz). Trimethylsilane was used with deuteriochloroform and 3-(trimethylsilyl)-1 propanesulfonic acid with deuterium oxide as internal references. ^{13}C-NMR spectra were recorded on a

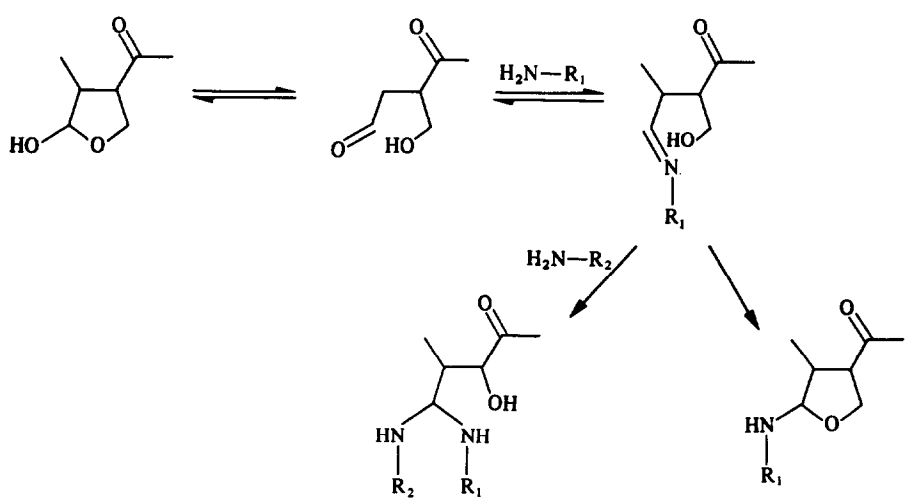


Fig. 2. Reaction mechanism proposed for the formation of the adducts between botryodiplodin and amino derivatives.

WP 80 Brucker spectrometer. Mass spectra were recorded on a Varian MAT 311 for high-resolution, and on a ZAB HF for the fast atom bombardment spectra. Ultraviolet spectra were run on a Beckman 24 spectrometer. Infrared spectra were obtained with a Perkin–Elmer 157 G spectrometer. All melting points are uncorrected. Botryodiplodin **1** and botryodiplodin acetate **2** were obtained as described by Moreau.[3] Synthesis of 1-propylcytosine and 9-propyladenine was as described by Léonard.[14]

2-Methoxy 3-methyl-4-acetyl tetrahydrofuran **3**

Botryodiplodin (100 mg) was dissolved in 700 μl of DMF. Silver oxide (300 mg) and methyl iodide (250 μl) were added, and the mixture was stirred for 1 hr at 20°. The mixture was subjected to a silica gel column eluted with ethyl acetate–n-hexane (30:70). Crude **3** was obtained and then purified by HPLC; 70 mg of a colourless oil of **3** were obtained.

High-resolution mass spectrum *m/e* 157.0862 $(M-H_2O)^+$: calc 157.0864, 98, 85, 83, 71. IR ($CHCl_3$): 1710 cm^{-1}. UV ($CHCl_3$): λ_{max} 278 nm (ε 45). ^{1}H-NMR ($CDCl_3$): δ 0.86 (d, 3H, d, J = 7.3 Hz); 2.19 (3H, s); 2.58 (1H, quint., J = 7.3 Hz); 3.33 (3H, s); 3.57 (1H, q, J = 7.3 Hz); 3.93 (1H, t, J = 7.3 Hz); 4.29 (1H, t, J = 7.3 Hz); 4.66 (1H, s). ^{13}C-NMR ($CDCl_3$): δ 12.5 (q); 29.5 (q); 42.0 (d); 53.3 (d); 54.0 (q); 66.3 (t); 110.7 (d); 204.3 (s).

N - [2 - (3 - *Methyl* - 4 - *acetyl)tetrahydrofuran*] 2 - *amino* - *pyrimidine* **4**

2-Aminopyrimidine (95 mg; 1 mmol) and botryodiplodin (144 mg; 1 mmol) were dissolved in 1 ml of methanol and stirred at 60° for 48 hr. The mixture was subjected to silica gel column chromatography, eluted with ethyl acetate–methanol (93:7). Compound **4** was then purified by HPLC using a semi preparative column (50 cm long, 1 cm i.d) of 5–20 μm Lichroprep (Merck). A crystallisation from ethyl ether–methanol gave 28 mg of **4**. $[\alpha]_D$ −25.1 (*c* = 7.65 mg/l, $CHCl_3$), m.p. 81–83°. High resolution mass spectrum *m/e* 221.1163 (M^+, calc 221.1164), 178, 124, 96, 95, 83. IR ($CHCl_3$): 3300–3500; 1710 cm^{-1}. UV ($CHCl_3$): λ_{max} 241 nm (ε 5800); 288 nm. ^{1}H-NMR ($CDCl_3$): δ 1.22 (3H, d, J = 7.3 Hz); 2.24 (3H, s); 2.44 (1H, sext., J = 7.3 Hz); 3.01 (1H, q, J = 7.3 Hz); 3.98 (1H, dd, J_1 = 7.3 Hz, J_2 = 8.6 Hz); 4.21 (1H, t, J = 8.6 Hz); 5.59 (1H, dd, J_1 = 9 Hz, J_2 = 7.3 Hz); 6.08 (1H, d, 9 Hz); 6.66 (1H, t, J = 4.7 Hz); 8.36 (2H, d, J = 4.7 Hz). ^{13}C-NMR ($CDCl_3$): δ 17.0 (q); 29.8 (s); 41.6 (d); 59.0 (d); 67.6 (t); 89.1 (d); 112.3 (d); 158.1 (d); 161.7 (s); 207.4 (s).

N - [2 - (3 - *Methyl* - 4 - *acetyl)tetrahydrofuran* 4 - *amino* - 2,6 - *dimethyl pyrimidine* **5**

2,6-Dimethyl 4-aminopyrimidine (123 mg; 1 mmol) and botryodiplodin (144 mg; 1 mmol) in 1 ml of methanol were stirred at 60° for 48 hr. Purification of **5** was achieved as described for **4**, and gave 28 mg of white crystals **5**. $[\alpha]_D$ −133 (*c* = 4.15 mg/ml, $CHCl_3$), m.p. 110–112°. High resolution mass spectrum *m/e* 249.1494 (M^+, calc 249.1477); 206; 179; 152; 124; 123; 83. IR ($CHCl_3$): 3300–3500, 1710 cm^{-1}. UV ($CHCl_3$): λ_{max} 242 nm (ε 5130); 268 nm (ε 4500). ^{1}H-NMR ($CDCl_3$): δ 1.21 (3H, d, J = 7.3 Hz); 2.24 (3H, s); 2.33 (3H, s); 2.40 (1H, m); 2.50 (3H, s); 3.01 (1H, m); 3.94 (1H, dd, J_1 = 8.5 Hz, J_2 = 7.3 Hz); 4.22 (1H, t, J = 3.5 Hz); 5.36 (1H, s); 5.90 (1H, d, J = 9 Hz); 6.21 (1H, s). ^{13}C-NMR ($CDCl_3$): δ 17.6 (q); 24.0 (q); 25.9 (q); 29.9 (q); 42.1 (d); 58.9 (d); 67.9 (t); 88.9 (d); 100.3 (d); 162.1 (s); 165.8 (s); 167.3 (s); 207.7 (s). Anal. calc for $C_{13}H_{19}N_3O_2$: C, 62.62; H, 7.68; N, 16.84. Found: C, 62.39; H, 7.70; N, 16.92%.

N - [2 - (3 - *Methyl* - 4 - *acetyl)tetrahydrofuran*] 1 - *propyl cytosine* **6**

The reaction was carried out with 153 mg (1 mmol) of 1-propyl cytosine, 144 mg (1 mmol) of botryodiplodin in 1 ml of methanol. The mixture was stirred at 60° for 48 hr. Purification of **6** was achieved as described above and crystallization in ethyl ether-methanol gave 33 mg of white crystals **6**. $[\alpha]_D$ −41.6 (*c* = 3.15 mg/ml, $CHCl_3$), m.p. = 132–134°. High resolution mass spectrum *m/e* 279.1581 (M^+, calc: 279.1582); 236; 209; 182; 154; 102; 83. IR ($CHCl_3$): 3300–3500; 1710 cm^{-1}. UV ($CHCl_3$): λ_{max} 285 nm; 248 nm. ^{1}H-NMR ($CDCl_3$): δ 0.92 (3H, t, J = 7.3 Hz); 1.19 (3H, d, J = 7 Hz); 1.76 (2H, m, J = 7.3 Hz); 2.25 (3H, s); 2.46 (1H, m); 2.99 (1H, m); 3.75 (2H, m, J = 7.3 Hz); 3.95 (1H, dd, J_1 = 8.6 Hz, J_2 = 5.6 Hz); 4.18 (1H, t, J = 8.6 Hz); 5.62 (1H, s); 5.89 (1H, s); 6.26 (1H, s); 7.22 (1H, s). ^{13}C-NMR ($CDCl_3$): δ 11.0 (q); 17.6 (q); 22.3 (t); 29.9 (q); 41.8 (d); 51.9 (t); 58.6 (d); 68.0 (t); 87.7 (d); 93.6 (d); 156.4 (d); 164.0 (s); 206.7 (s); 209.2 (s).

N - [2 - (3 - *Methyl* - 4 - *acetyl)tetrahydrofuran*] 9 - *propyl adenine* **7a,b**

Botryodiplodin (144 mg; 1 mmol) and 9-propyl adenine (177 mg; 1 mmol) were dissolved in 1 ml of methanol. The mixture was stirred at 60° for 48 hr. Purification of **7a,b** was achieved as described above. The separation of the two isomers was carried out by HPLC. A crystallization in ethyl ether gave 17 mg of white crystals, 11 mg of **7b** were obtained as a colourless oil.

Compound **7a**. M.p. 140–142°. High resolution mass spectrum *m/e* 303.1694 (M^+, calc: 309.1695); 260; 233; 218; 178; 177; 135; 83. IR ($CHCl_3$): 3300–3500; 1710 cm^{-1}. UV ($CHCl_3$): λ_{max} 268 nm (ε 15,200). ^{1}H-NMR ($CDCl_3$): δ 0.93 (3H, t, J = 7.3 Hz); 1.23 (3H, d, J = 7.3 Hz); 1.90 (2H, sext., J = 7.3 Hz); 2.24 (3H, s); 2.54 (1H, sext., J = 7.3 Hz); 3.03 (1H, t, d, J_1 = 7.3 Hz, J_2 = 8.5 Hz); 4.00 (1H, dd, J_1 = 7.3 Hz, J_2 = 8.5 Hz); 4.14 (2H, t, J = 7.3 Hz); 4.24 (1H, t, J = 8.5 Hz); 5.95 (1H, s); 6.86 (1H, s); 7.81 (1H, s); 8.44 (1H, s). ^{13}C-NMR ($CDCl_3$): δ 11.1 (q); 17.2 (q); 24.3 (t); 29.9 (q); 42.2 (d); 45.4 (t); 59.0 (d); 68.0 (t); 88.4 (d); 120.2 (s); 140.7 (d); 149.2 (s); 152.8 (d); 154.2 (s); 207.7 (s). Anal. calc for $C_{15}H_{12}N_5O_2$: C, 59.38; H, 6.98; N, 23.09. Found: C, 59.61; H, 7.03; N, 22.89%.

Compound **7b**. High resolution mass spectrum *m/e* 303.1694 (M^+, calc: 303.1695); 260; 233; 218; 178; 177; 135; 83. IR ($CHCl_3$): 3300–3500; 1710 cm^{-1}. UV ($CHCl_3$): λ_{max} 268 nm (ε 15,100). ^{1}H-NMR ($CDCl_3$): δ 0.96 (3H, t, J = 7.5 Hz); 1.12 (3H, d, J = 7.5 Hz); 1.93 (2H, sext., J = 7.5 Hz); 2.24 (3H, s); 2.68 (1H, quint. d, J = 7.5 Hz, J_2 = 3.5 Hz); 3.55 (1H, q, J = 7.5 Hz); 4.10 (1H, dd, J_1 = 7.5 Hz, J_2 = 9 Hz); 4.19 (2H, t, J = 7.5 Hz); 4.26 (1H, dd, J_1 = 7.5 Hz, J_2 = 9 Hz); 5.95 (1H, s); 6.36 (1H, d, J = 8 Hz); 7.80 (1H, s); 8.46 (1H, s). ^{13}C-NMR ($CDCl_3$): δ 11.1 (q); 12.9 (q); 23.4 (t); 30.5 (q); 42.4 (d); 45.5 (t); 54.0 (d); 67.0 (t); 88.6 (d); 119.8 (s); 140.4 (d); 149.0 (d); 152.9 (d); 153.8 (s); 206.5 (s).

N - [2 - (3 - *Methyl* - 4 - *acetyl)tetrahydrofuran*] 2' - *deoxyadenosine* **8**

Botryodiplodin (144 mg; 1 mmol) and 2'-deoxyadenosine monohydrate (269 mg; 1 mmol) were dissolved in 1 ml of 0.2 M citrate buffer pH 4.5 and stirred at 60° for 48 hr. The mixture was then purified by HPLC using a semi-preparative column (50 cm long, 1 cm i.d.) of RP-8 5–20 μm (Merck) and eluted with water–acetonitrile (80:20). 28 mg of **8** were obtained.

Mass spectrum (FAB positive) *m/e* 400 $(M+Na)^+$; 378 $(M+H)^+$; 262; 217; 207; 185; 136. IR (KBr): 3300–3500; 1710 cm^{-1}. UV (H_2O, pH 7) λ_{max} 211 nm (ε 13,550); 267 nm (ε 11,550). ^{1}H-NMR (D_2O): δ 1.28 (3H, d, J = 7 Hz); 2.36 (3H, s); 2.59 (2H, m); 2.79 (1H, m); 3.33 (1H, m); 3.84 (2H, m); 4.08 (1H, m); 4.26 (2H, m); 4.66 (1H, m); 5.71 (1H, s); 6.44 (1H, t); 8.26 (1H, s); 8.31 (1H, s). ^{13}C-NMR (D_2O): δ 16.3; 30.1; 40.0; 42.5; 58.9; 62.6; 68.1; 72.2; 85.6; 88.4; 89.3; 120.5; 140.7; 147.0; 153.1; 154.9; 214.9.

N-[2-(3-*Methyl*-4-*acetyl)tetrahydrofuran*] 2'-*deoxycytine* **9**

Botryodiplodin (144 mg; 1 mmol) and of 2'-deoxycytidine monohydrate (245 mg; 1 mmol) were dissolved in 1 ml of 0.2 M nitrate buffer pH 4.5, and stirred at 60° for 48 hr. The reaction mixture was then purified by gel permeation with a Bio-Rad P_2 column (50 cm long, 1 cm i.d.), eluted with water–methanol (90:10). 18 mg of a colourless oil were obtained and purified by HPLC using a semi-preparative column RP-8 eluted with water–acetonitrile (85:15). 15 mg of **9** were obtained.

Mass spectrum (FAB positive) *m/e* 354 $(M+H)^+$; 238; 195;

185; 115; 112. IR (KBr): 300–3500; 1710 cm^{-1}. UV (H_2O, pH = 7): λ_{max} 276 nm (ε 16,000); 238 nm (ε 13,450). ^{1}H-NMR (D_2O): δ 1.25 (3H, d); 2.31 (3H, s); 2.45 (3H, m); 3.37 (1H, m); 3.82 (2H, m); 4.07 (1H, m); 4.08 (1H, m); 4.18 (1H, m); 4.44 (1H, m); 5.45 (1H, s); 6.15 (1H, s); 6.26 (1H, t); 7.90 (1H, s). ^{13}C-NMR (D_2O): δ 16.2; 34.7; 40.9; 43.0; 59.2; 62.8; 68.7; 72.0; 87.8; 88.3; 88.4; 98.4; 149.1; 158.9; 214.6; 227.0.

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