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BIOLOGICAL ACTIVITY OF SOME SYNTHETIC GIBBERELLIN GLUCOSYL ESTERS

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Key Word Index—Gibberellins; glucosyl esters of GA_1, GA_3, GA_4, GA_{37} and GA_{38}; synthesis; dwarf rice seedling bioassay; dwarf maize bioassay.

Abstract—*Four gibberellin (GA$_1$, GA$_3$, GA$_4$ and GA$_{37}$) glucosyl esters were synthesized and found to be as* active as their respective free acids in the rice seedling bioassay. The rapid hydrolysis of the glucosyl esters in rice seedlings was demonstrated by feeding experiments with glucosyl esters of GA_1 and GA_4.

INTRODUCTION

WE HAVE previously reported the isolation and characterization of four gibberellin glucosyl esters, namely, glucosyl esters of GA_1, GA_4, GA_{37} and GA_{38}, from mature seeds of *Phaseolus vulgaris*.[1,2] The present paper describes the synthesis, structural confirmation and biological properties of four gibberellin glucosyl esters.

RESULTS AND DISCUSSION

Methods for the preparation of the acetylglucosyl ester of GA_3 have been reported by two groups[3,4] and we used a modification of the former to prepare the acetylglucosyl esters of GA_1, GA_3, GA_4 and GA_{37}. The conditions for the hydrolysis of the acetoxy groups in the glucose moiety are very critical because treatment with alkali may cause rearrangement of ring-A of GA_3 and the partial epimerization of the C-3 hydroxyl group in C-3 hydroxy gibberellins. Successful deacetylation was achieved by treatment with *ca* 0·02 N sodium methoxide in methanol at $-10°$ for 1 hr. Under these conditions, the acetylglucosyl esters of GA_1, GA_3, GA_4 and GA_{37} were hydrolysed to their respective glucosyl esters in 40–60% yield. Prolonged treatment or an elevated temperature reduced the yield due to the formation of considerable amounts of gibberellin methyl esters. Of the gibberellin glucosyl esters prepared, only GA_3 glucosyl ester was crystalline, m.p. 217–219°. The remainder being semicrystalline solids. Their R_f values on TLC and R_t on GLC are listed in Table 1 and their NMR chemical shifts in Table 2. The GA_{37} glucosyl ester contained *ca* 20% of the isomer of GA_{37} (**1**), which was formed during acetylglucosylation; the reason for this isomerization was not clear. The synthetic GA_1 glucosyl ester was identical with

[1] HIRAGA, K., YOKOTA, T., MUROFUSHI, N. and TAKAHASHI, N. (1972) *Agr. Biol. Chem.* **36**, 345.
[2] HIRAGA, K., YOKOTA, T., MUROFUSHI, N. and TAKAHASHI, N. (1974) *Plant Growth Substances* 1973 (the Organizing Committee of the 8th Int. Conf. on Plant Growth Substances, eds.) In press.
[3] SCHREIBER, K., WEILAND, J. and SEMBDNER, G. (1969) *Tetrahedron* **25**, 5541.
[4] KEAY, P. J., MOFFATT, J. S. and MULHOLLAND, T. P. C. (1965) *J. Chem. Soc.* 1605.

the naturally occurring compound in all respects. Although glucosyl esters of GA_4 and GA_{37} were obtained only as a mixture from seeds, a comparison of the NMR spectra of the mixture and the MS and GLC R_t of their TMSi derivatives with those of the synthetic samples showed that the structural assignment of the naturally occurring glucosyl esters was correct.

TABLE 1. TLC R_f VALUES AND GLC R_t (min) OF GIBBERELLIN GLUCOSYL ESTERS

Compound	R_f* (a)	R_f* (b)	R_t (min) TMS derivative† (a)	R_t (min) TMS derivative† (b)
A_1GE	0·38	0·19	14·8	18·8
A_3GE	0·38	0·17	16·5	20·7
A_4GE	0·29	0·33	13·1	14·7
A_{37}GE	0·29	0·35	22·0	23·0
A_{38}GE	0·38	0·12	25·7	28·0

GE: glucosyl ester.
* Adsorbent: Silica gel G (a) $CHCl_3$–MeOH (3:1) (b) C_6H_6–Me_2CO (1:5).
† (a) 2%QF-1 (3 mm × 1 m), column temp. 224°, carrier gas N_2 (34 ml/min). (b) 2%OV-1 (3 mm × 1 m), column temp. 243°, carrier gas N_2 (33 ml/min).

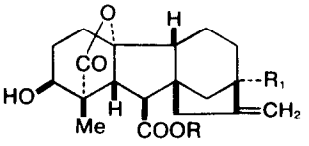
TABLE 2. 100 MHz NMR SPECTRA OF GIBBERELLIN GLUCOSYL ESTERS

Compound	H-1	H-2	4-Me (*s*)	H-5 (*d*)	H-6 (*d*)	H-17 (*s*)	H-20 (*d*)	H-1′ (*d*)
A_1GE	*	*	1·11	2·70 (*J*10)	3·25 (*J*10)	4·90 5·20	—	5·54 (*J*8)
A_3GE	6·41 (*d*, *J*10)	5·92 (*q*, *J*4,10)	1·19	2·82 (*J*11)	3·28 (*J*11)	4·94 5·22	—	5·56 (*J*8)
A_4GE	*	*	1·10	2·70 (*J*12)	3·27 (*J*12)	4·86 4·96	—	5·52 (*J*8)
A_{37}	*	*	1·17	2·82	2·82	4·82 4·92	4·12 (*J*13·5) 4·49 (*J*13·5)	5·53 (*J*7·5)
A_{38}GE	*	*	1·16	2·82	2·82	4·83 5·15	4·16 (*J*13) 4·55 (*J*13)	5·55 (*J*8)

GE: glucosyl ester. Chemical shifts and coupling constants (*J*) are expressed in δ-values and Hz respectively. Solvent: d_6-Me_2CO-D_2O.
* These signals could not be distinguished from overlapping signals.

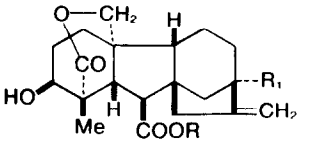
The biological activities of the glucosyl esters of GA_1, GA_3, GA_4, GA_{37} and GA_{38}, together with their respective free acids and methyl esters, were examined using the rice seedling (Tan-ginbozu and Waito-C) and the dwarf maize mutant (d_1 and d_5) bioassay. The results are summarized in Tables 3, 4 and 5, respectively. In the rice seedling tests using the water culture and micro drop method under non-sterile conditions, most gibberellin glucosyl esters were almost as active as the respective free gibberellins, whilst their methyl esters showed very low activities. In the dwarf maize test, gibberellin glucosyl esters were less active than their free acids, although the activities of gibberellin glucosyl esters

were greater than those of the methyl esters. These results are in marked contrast with previous results which showed that gibberellin glucosides had very low activities in comparison with their free acids using the same bioassays.[5,6] This difference may be explained by the facile hydrolysis of gibberellin glucosyl esters in the tissue of higher plants. It should be noted that gibberellin glucosyl esters are less active in the dwarf maize assay than in the dwarf rice micro drop assay, suggesting that the enzyme system hydrolysing glucosyl esters in maize is different from that in rice.



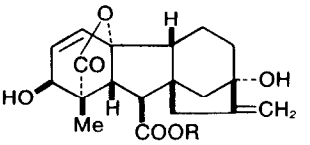
A_1 Glucosyl ester
R = β-D-glucosyl, R_1 = OH

A_4 Glucosyl ester
R = β-D-glucosyl, R_1 = H

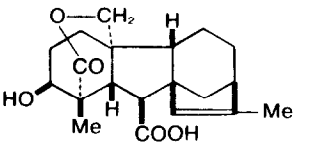


A_{37} Glucosyl ester
R = β-D-glucosyl, R_1 = H

A_{38} Glucosyl ester
R = β-D-glucosyl, R_1 = OH



A_3 Glucosyl ester
R = β-D-glucosyl



(1)

TABLE 3. ACTIVITY OF GIBBERELLIN GLUCOSYL ESTERS ON DWARF RICE IN THE WATER CULTURE ASSAY

	Tan-ginbozu			Waito-C			
μM/ml	10^{-4}	10^{-3}	10^{-2}	10^{-4}	10^{-3}	10^{-2}	Control
A_1GE	19·4 ± 1·0	29·2 ± 1·7	80·9 ± 3·4	20·6 ± 0·7	31·0 ± 2·3	88·2 ± 5·8	a
A_1	22·9 ± 1·7	39·6 ± 0·9	94·7 ± 3·4	20·7 ± 0·3	44·2 ± 1·7	137·1 ± 8·6	a
A_1Me	27·0 ± 1·9	26·2 ± 1·7	26·9 ± 1·3	19·6 ± 0·6	26·2 ± 0·4	27·8 ± 0·8	a
A_3GE	27·6 ± 1·9	55·2 ± 2·2	135·8 ± 8·9	27·1 ± 1·3	83·6 ± 5·1	179·1 ± 10·4	a
A_3	34·8 ± 1·6	57·5 ± 2·5	114·0 ± 5·7	33·5 ± 1·0	96·4 ± 3·0	138·1 ± 7·6	a
A_3Me	22·4 ± 1·2	24·5 ± 1·1	25·8 ± 1·1	22·9 ± 0·8	25·8 ± 0·5	32·9 ± 1·8	a
A_4GE	20·1 ± 1·3	27·7 ± 0·9	62·8 ± 6·3	19·0 ± 0·7	23·1 ± 0·6	71·5 ± 7·4	a
A_4	20·2 ± 0·6	25·5 ± 0·7	64·0 ± 5·0	19·7 ± 0·8	21·7 ± 0·7	58·4 ± 4·2	a
A_4Me	19·1 ± 0·7	20·9 ± 0·5	26·4 ± 1·9	19·2 ± 0·5	24·8 ± 0·8	30·0 ± 1·6	a
A_{37}GE	26·5 ± 5·1	30·0 ± 0·7	50·6 ± 7·0	22·0 ± 0·2	39·6 ± 1·6	58·5 ± 1·3	b
A_{37}	20·5 ± 0·7	34·3 ± 0·7	59·8 ± 1·9	23·7 ± 0·6	43·6 ± 1·7	71·0 ± 3·2	b
A_{38}GE	20·1 ± 0·6	29·3 ± 1·4	42·1 ± 3·3	21·3 ± 0·7	33·5 ± 0·9	51·0 ± 2·1	b
A_{38}	19·3 ± 0·6	26·3 ± 1·2	42·3 ± 1·6	20·2 ± 0·5	32·9 ± 0·6	45·5 ± 1·5	b
Control a		14·6 ± 0·5			18·9 ± 0·4		
Control b		17·7 ± 0·4			15·5 ± 0·6		

GE: glucosyl ester, Me: methyl ester. Each value represents the mean length (mm) of the 2nd leaf sheath and its standard error (n = 5–7).

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TABLE 4. ACTIVITY OF GIBBERELLIN GLUCOSYL ESTERS ON DWARF RICE IN THE MICRO DROP ASSAY

mμM/plant	Tan-ginbozu 10^{-3}	Tan-ginbozu 10^{-2}	Tan-ginbozu 10^{-1}	Waito-C 10^{-3}	Waito-C 10^{-2}	Waito-C 10^{-1}	Control
A_1GE	21·5 ± 0·7	73·2 ± 0·7	28·9 ± 1·0	18·0 ± 0·2	21·2 ± 0·6	27·8 ± 1·2	a
A_1	20·2 ± 1·0	24·1 ± 0·8	32·3 ± 0·7	19·3 ± 0·4	21·1 ± 0·4	29·6 ± 1·0	a
A_1Me	20·2 ± 0·7	21·6 ± 0·4	25·2 ± 1·2	19·4 ± 0·4	20·3 ± 0·2	23·9 ± 0·3	a
A_3GE	22·3 ± 0·9	33·4 ± 0·5	42·6 ± 0·5	21·6 ± 0·4	33·0 ± 0·8	48·5 ± 1·8	a
A_3	24·8 ± 0·4	34·2 ± 1·6	45·6 ± 1·1	22·2 ± 0·5	36·4 ± 1·6	46·5 ± 1·6	a
A_3Me	19·3 ± 0·9	21·0 ± 0·6	22·4 ± 0·7	19·1 ± 0·4	20·8 ± 0·6	24·3 ± 0·3	a
A_4GE	21·3 ± 0·7	23·4 ± 0·4	27·9 ± 0·9	18·7 ± 0·3	19·0 ± 0·5	23·4 ± 0·8	a
A_4	21·8 ± 0·4	23·6 ± 1·1	25·1 ± 1·4	19·6 ± 0·4	22·2 ± 0·4	32·0 ± 2·2	a
A_4Me	21·1 ± 0·4	21·5 ± 0·9	23·5 ± 0·6	18·3 ± 0·5	19·0 ± 0·1	21·1 ± 0·6	a
A_{37}GE	19·5 ± 0·5	21·6 ± 0·9	22·7 ± 1·9	17·7 ± 0·2	18·8 ± 0·4	24·7 ± 1·5	b
A_{37}	17·5 ± 0·5	23·0 ± 0·4	27·3 ± 1·1	17·8 ± 0·4	18·6 ± 0·7	28·2 ± 0·4	b
A_{38}GE	18·5 ± 0·4	20·3 ± 0·7	31·3 ± 1·4	20·4 ± 1·0	21·7 ± 0·8	29·4 ± 1·0	b
A_{38}	19·8 ± 0·5	20·7 ± 0·5	28·1 ± 1·5	18·1 ± 0·3	19·7 ± 0·6	24·4 ± 1·2	b
Control a		21·3 ± 0·8			18·5 ± 0·2		
Control b		18·1 ± 0·8			17·8 ± 0·4		

GE: glucosyl ester, Me: methyl ester. Each value represents the mean length (mm) of the 2nd leaf sheath and its standard error (n = 5–7).

TABLE 5. ACTIVITY OF GIBBERELLIN GLUCOSYL ESTERS IN THE DWARF MAIZE ASSAY

μg/plant	Dwarf maize d_1 0·1	Dwarf maize d_1 1	Dwarf maize d_1 10	Dwarf maize d_5 0·1	Dwarf maize d_5 1	Dwarf maize d_5 10
A_1GE	47·0 ± 2·9	61·3 ± 1·8	94·8 ± 4·3	46·0 ± 1·7	61·8 ± 2·4	86·5 ± 2·6
A_1	61·0 ± 3·1	101·0 ± 8·0	129·0 ± 8·9	68·5 ± 4·1	86·0 ± 0·6	126·7 ± 2·7
A_1Me	46·8 ± 2·4	43·7 ± 1·5	43·8 ± 3·2	47·5 ± 1·7	44·0 ± 1·7	54·8 ± 3·4
A_3GE	58·3 ± 2·3	86·7 ± 8·6	110·0 ± 6·4	55·0 ± 1·7	82·0 ± 4·5	109·3 ± 5·6
A_3	69·3 ± 1·3	94·0 ± 3·7	132·5 ± 10·6	66·4 ± 3·6	97·6 ± 4·9	115·7 ± 1·0
A_3Me	42·5 ± 2·6	47·0 ± 0·7	54·8 ± 3·0	41·9 ± 1·3	47·6 ± 2·6	47·6 ± 2·8
A_4GE	53·5 ± 2·0	77·3 ± 0·6	98·8 ± 5·0	57·5 ± 1·7	64·3 ± 5·2	85·6 ± 1·6
A_4	44·5 ± 0·5	53·0 ± 1·0	112·0 ± 2·7	51·1 ± 1·8	55·0 ± 1·6	103·7 ± 0·9
A_4Me	—	—	—	39·5 ± 0·6	42·2 ± 1·7	47·2 ± 2·4
A_{37}GE	45·5 ± 5·5	56·0 ± 2·9	72·3 ± 3·8	45·4 ± 1·6	53·6 ± 1·3	69·2 ± 2·8
A_{37}	40·8 ± 2·1	55·8 ± 3·7	104·6 ± 4·1	45·1 ± 1·2	58·9 ± 3·0	112·9 ± 4·2
A_{38}GE	46·3 ± 3·1	60·5 ± 3·2	73·5 ± 6·0	54·7 ± 2·9	60·7 ± 1·7	69·2 ± 2·2
A_{38}	59·3 ± 2·7	72·3 ± 3·6	93·0 ± 7·8	49·3 ± 0·6	71·0 ± 3·2	81·3 ± 6·0
Control		31·6 ± 1·3			35·5 ± 1·0	

GE: glucosyl ester, Me: methyl ester. Each value represents the mean sum (mm) of the 1st and 2nd leaf sheath length and its standard error (n = 4).

To confirm the rapid hydrolysis of gibberellin glucosyl esters in rice seedlings, glucosyl esters of 1,2-[^{3}H]GA_1 and 1,2-[^{3}H]GA_4 were prepared and fed to rice seedlings by the

micro drop method.[7] At given intervals during the culture period, treated plants were harvested and extracted with methanol. The extract was fractionated into an acidic ethyl acetate (AE), a neutral ethyl acetate (NE), an acidic butanol (AB) and a neutral (NB) fraction, and the radioactivity in each fraction determined. These results are summarized in Table 6. Usually, free gibberellins are partitioned into the AE fraction, GA_4 glucosyl ester into the NE fraction, GA_1 glucosyl ester into the NB fraction and other gibberellin glucosides into the AB fraction. Rapid hydrolysis of GA_4 glucosyl ester was indicated by the decrease of radioactivity in the NE fraction and a marked increase in the AE fraction after 12 hr incubation. In the case of GA_1 glucosyl ester, the rate of hydrolysis was rather slower than in the case of GA_4 glucosyl ester but most of GA_1 glucosyl ester was hydrolysed after 48 hr.

TABLE 6. CHANGES IN THE QUANTITIES OF GIBBERELLIN GLUCOSYL ESTERS IN DWARF RICE SEEDLINGS

Source and fraction	Time (hr) 5	12	24	48	72
	Distribution of radioactivity in each fraction %				
A_1 glucosyl ester					
Recovery total (%)	35·2	27·7	13·7	13·4	12·7
Acid EtOAc	25·2	38·2	44·0	52·3	38·8
Neutral EtOAc	3·5	3·1	3·5	3·4	3·6
Acid BuOH	21·1	22·1	16·4	21·2	35·4
Neutral BuOH	37·8	27·3	28·8	15·8	17·2
Aq. residue	12·4	9·3	7·3	7·3	5·0
A_4 glucosyl ester					
Recovery total (%)	43·8	33·3	15·0	16·4	14·8
Acid EtOAc	34·5	55·7	52·8	24·9	29·5
Neutral EtOAc	41·9	16·2	8·6	13·5	19·1
Acid BuOH	5·1	8·6	26·3	44·6	34·9
Neutral BuOH	14·8	17·3	9·4	14·0	11·6
Aq. residue	3·7	2·2	2·9	3·0	4·9

Each value represents the recovery yield and the distribution of radioactivity in each fraction (%).

This experiment clearly confirmed the facile hydrolysis of gibberellin glucosyl esters in rice seedlings. A similar experiment showed that the hydrolysis of glucosyl esters was more complicated in dwarf maize than in rice seedlings.

The facile hydrolysis of synthetic gibberellin glucosyl esters by plant tissues suggests that the endogenous glucosyl esters of mature seeds probably release free active gibberellins during germination on activation of a hydrolytic enzyme system.

EXPERIMENTAL

Preparation of gibberellin glucosyl esters. Free gibberellin (150 mg) was dissolved in dry dioxane (10 ml) and α-bromoacetoglucose (190 mg), Ag_2O (250 mg) and a few pieces of molecular sieve were added to the soln. The mixture was stirred at 25° in darkness. After 20 hr, solids were filtered off and the cake was washed with EtOAc (20 ml) several times and the combined filtrate was extracted twice with aq. $NaHCO_3$. The organic phase was dried and evaporated. The solid thus obtained was crystallized from EtOAc-hexane to give gibberellin acetylglucosyl ester (120–180 mg) as fine needles. Physical data for the gibberellin acetylglucosyl esters are as follows (GA_{37} acetylglucosyl ester was not crystallized) A_1 M^+ 678 m.p. 186·5–188°, A_3 M^+ 676 m.p. 216–217°, A_4 M^+ 662 m.p. 185–187°. Gibberellin acetylglucosyl ester (60 mg) was dissolved in MeOH (7 ml) and the soln was cooled to −10°. To this soln 0·05 M NaOMe (5 ml) was added and the mixture stirred at −10° for 1 hr. 0·1 M HCl (2·5 ml) was added and the soln evaporated under red. pres. at 30°. The solid thus obtained was extracted with Me_2CO–EtOH (1:1) at 35–40° several times. On evaporation of solvent, crude gibberellin glucosyl ester (40 mg) was obtained. Prep. TLC ($CHCl_3$–MeOH, 3:1) afforded the pure gibberellin glucosyl ester (25 mg).

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[^{3}H]GA_1 was prepared from GA_3 methyl ester (600 mg) by hydrogenation[8] with H_2 enriched with ^{3}H over 2% Pd on $BaCO_3$ (700 mg) partially poisoned with C_5H_5N in EtOAc. The neutral and acidic fractions were separated by solvent extraction. The neutral fraction was purified on an alumina column[9] to give [^{3}H]GA_1 methyl ester (70 mg). The acidic fraction was crystallized from EtOAc–hexane to give the reduction product (300 mg). The reduction product was dissolved in Me_2CO (30 ml) and 3 M HCl (15 ml) was added and the mixture heated at 65–70° for 6 hr. The neutral fraction recovered from the reaction mixture was purified on an alumina column to give [^{3}H]GA_1 methyl ester (50 mg). The combined [^{3}H]GA_1 methyl ester was hydrolysed using the method of Bartlet and Johnson[10] with some modifications. Propyl mercaptane (2 ml) in HMPA (3 ml) was added to a suspension of lithium hydride (0·3 g) and HMPA (5 ml). After 30 min, [^{3}H]GA_1 methyl ester (120 mg) in HMPA (6 ml) was added to the suspension with stirring under N_2. After 1·5 hr, the reaction mixture was poured into acidified ice H_2O and the aq. soln extracted with EtOAc. The EtOAc was extracted with aq. $NaHCO_3$ and the aq. phase washed with EtOAc several times. After acidification, the aq. phase was extracted with EtOAc; evaporation of the EtOAc gave a semicrystalline solid. TLC purification on silica gel using EtOAc–$CHCl_3$–HOAc, 20:8:1) and successive crystallization yielded [^{3}H]GA_1 (60 mg, 2 mCi/mmol). This sample (10 mg) was used for the preparation of GA_1 glucosyl ester. In the purification process, an addition of cold GA_1 acetylglucosyl ester was made, yielding [^{3}H]GA_1 glucosyl ester with a final radioactivity of 13·2 μCi/mmol.

[^{3}H]GA_4 was glucosylated to give [^{3}H]GA_4 glucosyl ester with a radioactivity of 0·16 μCi/mmol.

(GLC) R_t were determined using an FID instrument. Silanized glass columns, 1 m × 3 mm were packed with 2% OV-1 or 2% QF-1 on silanized Chromosorb W. Dry samples of gibberellin glucosyl esters were dissolved in dry C_5H_5N–BSA–trimethylsilyl chloride (1:2:1). After standing for 5 min, the solns (1 μl) were injected.

NMR spectra were determined on a 100 MHz instrument in $CDCl_3$.

MS were obtained at 70 eV using a direct inlet system and a chamber temp of 250°.

Dwarf rice test. Rice seeds (*Oryza sativa* L.) dwarf cv. Tan-ginbozu and Waito-C were used for the assay. The H_2O culture assay was carried out using the method of Yokota *et al.*[5] under non-sterile conditions and the micro drop assay using that of Murakami.[7]

Dwarf maize test. The assay was conducted according to the method of Yamane *et al.*[6] *Zea mays* L., mutant d_1 and d_5 were used for the assay.

Feeding experiments. The micro drop method was used for the application of ^{3}H-labelled gibberellin glucosyl esters. An aliquot (1 μl) of 30% aq. Me_2CO soln containing [^{3}H]GA_1 glucosyl ester was applied to each of 7 rice seedlings (Tan-ginbozu, *ca* 0·6 μg per plant). In the same way *ca* 8·6 μg of [^{3}H]GA_4 glucosyl ester was applied to each of 14 rice seedlings. After 5, 12, 24, 48 and 72 hr the plants were removed from agar and extracted with MeOH. After evaporation of the solvent *in vacuo*, the aq. soln was adjusted to pH 2·5 with 20% H_2SO_4 and extracted with EtOAc, and then *n*-BuOH. The EtOAc and BuOH layers were extracted with aq. $NaHCO_3$, to give the respective neutral EtOAc (NE) and neutral BuOH (NB) fraction. The aq. phases were re-extracted with EtOAc and BuOH, respectively, at pH 2·5 to give an acidic ethyl EtOAc (AE) and an acidic BuOH (AB) fraction. Radioactivity of each fraction was determined using a liquid scintillation spectrometry and Bray's solution as the scintillator.

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