MICROBIAL TRANSFORMATIONS OF NATURAL ANTITUMOR AGENTS. 20. GLUCOSYLATION OF VIRIDICATUMTOXIN

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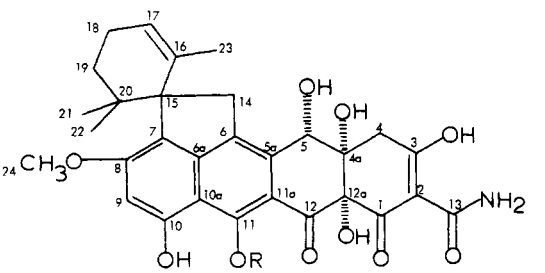
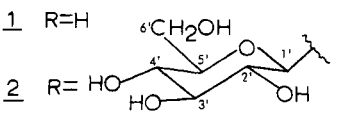
ABSTRACT.—Microbial transformation studies were performed with viridicatumtoxin. Microorganisms of the genera *Absidia*, *Cunninghamella*, *Cyathus*, *Penicillium*, *Rhizopus* and *Stemphylium* all produced a common metabolite. The compound was produced in preparative scale by a *Rhizopus* strain and was identified as viridicatumtoxin-11-*O*-β-glucoside.

Microbial transformation experiments have been conducted with a wide variety of antitumor compounds for the purpose of producing novel and useful derivatives which are difficult to prepare by synthetic methods (1–3). Viridicatumtoxin (NSC #159628) (**1**) is a fungal toxin isolated from *Penicillium viridicatum* Westling, and its structure was obtained by X-ray crystallographic methods in 1976 (4). The compound, which has also demonstrated modest antitumor activity, was provided by the National Cancer Institute for biotransformation study. An examination of the structure of viridicatumtoxin suggests that it would be a rich substrate for microbial modification, and expected reactions would include *O*-demethylation, amide hydrolysis, dehydration, a variety of hydroxylation reactions, and reductions. This report is concerned with the production, isolation, and identification of the major and most common microbial transformation product as viridicatumtoxin-11-*O*-β-glucoside.





EXPERIMENTAL

GENERAL METHODS.—Infrared spectra were taken with a Beckman 4240 spectrophotometer, and uv spectra were determined with a model SP1800 Phillips Pye Unicam instrument. Melting points were determined in open ended capillary tubes on a Thomas Hoover apparatus. Mass spectra were determined on a Finnigan model 3200 gc/mass spectrometer with either a direct inlet probe or gc introduction of samples. Carbon-13 nmr spectra were obtained with a Bruker HX-90E spectrometer operating at 22.63 MHz with 0.2M solutions in d_6-DMSO. Proton nmr spectra were recorded on either a Varian T-60 in continuous wave mode or by Bruker HX-90E and JEOL FX-90Q spectrometers with FT methods.

CHROMATOGRAPHY.—Analytical thin-layer chromatography (tlc) was performed on 0.25 mm layers of phosphate-buffered silica gel plates. To prepare the plates, 25 g of silica gel GF_{254} (Merck) was slurried in 50 ml of 1/15M, pH 6.5 phosphate buffer and spread on the glass plates with a Quickfit Industries (London) spreader. The plates were air-dried and activated in an oven at 110° for 30 minutes and stored in an airtight storage cabinet. Plates were developed in a mobile phase consisting of chloroform-methanol-water-acetic acid (glacial) (90:20:2:1). Viridicatumtoxin (**1**) (Rf 0.6) and its metabolite (**2**) (Rf 0.3) were visible as bright yellow and yellow green spots, respectively, under 366 nm uv light. Preparative layer tlc was performed

on 0.75 mm thick layers consisting of 1:1 silica gel GF_{254} and Kieselguhr G (Merck) by the same phosphate buffer slurrying technique. After air drying, preparative layer plates were activated in an oven at 110° for 1.5 hr and stored in an airtight tank saturated with an atmosphere of methyl ethyl ketone saturated with water. Preparative tlc plates were developed twice with chloroform-water-methanol mixtures of 93:7:1.5 and 90:10:1.5, respectively, to separate the polar metabolite from starting material and other impurities.

High performance liquid chromatography was performed with a Waters ALC/GPC 202 instrument equipped with a M6000 solvent delivery system, an U6K Universal Injector and a 254 nm differential UV detector. The purity of metabolite preparations was checked on a reversed phase C-18 μ-bondapak (Waters) column (.4 x 25 cm) with acetonitrile-water-1/15 M, pH 2.0 phosphate buffer (33:56:6.5) at an average flow rate of 1.5 ml per min at an operating pressure of 1,500 psi. Samples (4 μl) in methanol were injected for analyses. Retention volumes of viridicatumtoxin (**1**) and the glucoside metabolite (**2**) were 12.6 ml and 4.5 ml, respectively, under these conditions.

Gas chromatography was performed on a Hewlett Packard model 5710 instrument equipped with a flame ionization detector with either OV-101 or OV-17 (3 ft) columns under operating conditions of 30 ml/min helium. Temperatures of the injector, columns and flame ionization detector were held nominal at 190°, 170° and 200°, respectively. Normally 1–2 μl samples were injected for analysis.

SILYLATION AND GLC–MASS SPECTRAL ANALYSIS OF SUGARS (5–7).—Samples of standard sugars, (0.2 mg), and those obtained from acidic or enzymatic hydrolysis of viridicatumtoxin-11-*O*-glucoside (**2**), were placed in a 1 ml vial tightly fitted with a rubber septum and 0.2 ml of silylating mixture (Sigma Sil-A containing trimethylchlorosilane, hexamethyldisilazone and pyridine 1:3:9) was added. The vials were left at room temperature with occasional shaking for 30 min when 1 μl aliquots were withdrawn and injected into the gas chromatograph. The sugar derivative derived from hydrolysis of the metabolite (**2**) and D-glucose gave the same retention times of 7.2 and 11.2 min (*alpha*- and *beta*-isomers) on OV-101, and co-injection of silyl derivatives of the unknown sugar and D-glucose resulted in intensification of the same peaks. GC/mass spectral analysis of authentic D-glucose and the sugar obtained by hydrolysis of the metabolite gave similar retention times and fragmentation patterns (7) as follows: *m/e* (% rel abundance) 435 (0.2), 345 (0.4), 319 (0.4), 317 (0.5), 305 (0.8), 291 (0.3), 243 (1.3), 231 (1.9), 217 (16.1), 204 (100), 191 (44.7), 189 (4.4), 169 (1.5), 157 (1.1), 147 (7.1), 143 (1.6), 129 (10.2), 117 (2.7), 103 (4.8), 73 (27.7).

PAPER CHROMATOGRAPHY OF SUGARS.—Approximately 5–10 μg quantities of standard sugars, including D-glucose, L-arabinose, D(−)-ribose, L-rhamnose, galactose, galacturonic acid, glucuronic acid, glucuronic acid lactone, and mannuronic acid lactone, were spotted onto Whatman No. 1 paper (15 x 23 cm) along with enzymatic and chemical hydrolysis products of the metabolite (**2**) which was then developed in ascending fashion with a solvent mixture of *n*-butanol-water-ethanol (20:11:5). Spots were visualized with a solution of aniline hydrogen phthalate and sprayed chromatograms were heated in an oven at 110° for 5–8 minutes. This revealed the strong similarity in color formed and in chromatographic mobility of the unknown product of hydrolysis of the metabolite and D-glucose. Co-chromatography with glucose in the same system revealed a single spot.

VIRIDICATUMTOXIN.—Viridicatumtoxin (NSC No. 159628) was obtained from the National Cancer Institute for study, and the properties of this sample were compared with literature values (4) as follows: mp 210–212° (lit 211°); ir (KBr disc) cm^{-1} 3500, 3400, 3240 (sh), 2960, 2940, 2920, 1625, 1590, 1575; uv (MeOH) nm (log ε) 239 (4.5798), 294 (4.602), 319 (3.4314), 332 (3.356), 350 (3.161) and 429 (4.000); ^{1}H-nmr ($CDCl_3$) ppm, 9.08 (broad s, 1H, exchange with D_2O), 8.69 (broad s, 1H, exchange with D_2O), 6.64 (s, 1H, 9–H), 6.05 (b, 1H, exchange with D_2O), 5.50 (broad s, 1H, 17–H), 4.51 (broad s, 1H, 5–H), 3.86 (s, 3H, OCH_3), 3.47 (s, 3H, OH's, exchanges with D_2O), 3.40 (s, 2H, 14–H), 2.70 (s, 2H, 4–H), 2.17 (m, 2H, 18–H), 1.65 (m, 2H, 19–H), 1.51 (s, 3H, 23–CH_3), 0.91 (s, 3H, 21– or 22–CH_3) and 0.47 (s, 3H, 21– or 22–CH_3); mass spectrum *m/e* 565 $(M)^+$, 548 $(M-NH_3)^+$; 529 $(M-2H_2O)^+$; 509 $(M-C_4H_8)^+$; 491 $(M-[C_4H_8+NH_3])^+$; ^{13}C-nmr ($CDCl_3$:CD_3OD,1:1), ppm (See table 1).

FERMENTATION PROCEDURE.—Fermentation methods used in the cultivation of microorganisms have been described (3). A two-stage incubation procedure was employed, and a solution of substrate viridicatumtoxin (**1**) in dimethylformamide (1 gm/20 ml) was added to 24-hr-old Stage II cultures at a final concentration of 0.2 mg/ml. of culture medium. Screening fermentations were conducted in steel-capped, 125 ml Delong culture flasks holding 25 ml of medium, while preparative scale incubations were conducted in 1 liter Delong flasks containing 150 ml of medium. All cultures were incubated at 25° with shaking on New Brunswick Scientific G–25 Gyrotory shakers operating at 250 rpm.

Screening experiments were conducted with 220 microorganisms, and 4 ml samples of viridicatumtoxin containing fermentations were taken at 12, 24, 72, 144 and 192 hours following the addition of substrate. Samples were adjusted to pH 4.0 with 2N HCl and extracted with 1 ml of ethyl acetate-n-butanol (9:1), and approximately 25 μl of each extract was spotted onto silica gel tlc plates. To confirm results, experiments were repeated with controls to verify that the products observed in fermentations were enzymatic transformation products and not merely artifacts arising from incubation conditions. Controls consisted of 5 mg of viridicatumtoxin added to sterile medium; to buffers at pH 3.6 (1/15 M sodium acetate), pH 5.0, 7.0 and 9.0 (1/15 M phosphate); and also cultures containing no substrate. Tlc examination of repeat incubations and controls revealed that viridicatumtoxin was decomposed only at pH 9.0, a condition which was not obtained in cultures forming any of the viridicatumtoxin metabolites. Very slight decomposition of the substrate occurred at pH 7.0 at one week of incubation.

PRODUCTION OF (2) FROM VIRIDICATUMTOXIN BY RHIZOPUS SPECIES 224.—Viridicatumtoxin (425 mg) was dissolved in 6 ml of dimethylformamide and evenly distributed among 17, 1-liter Delong flasks each containing 150 ml of 24 hr old Stage II culture. The course of metabolite formation was monitored by sampling at various time intervals. The metabolite could be detected within 24 hours, and the fermentation was terminated at 11 days when complete utilization of viridicatumtoxin had apparently occurred.

Multilayered cheesecloth was used to separate the cells from fermentation beers, and the filtrate and cells were worked up separately. The filtrate was adjusted to pH 4.2 with 2N HCl and exhaustively extracted with 19:1 ethyl acetate-*n*-butanol. The cells were subjected to Soxhlet extraction done with the same organic solvent mixture. Since compositions of cell and medium extracts were similar, they were combined, dried over anhydrous Na_2SO_4 and concentrated at room temperature to a viscous brown residue (2.0 g). When this residue was subjected to preparative tlc, it yielded 290 mg of (2) which was 95% pure by hplc analysis. Further attempts to purify this material by preparative hplc, tlc, or column chromatography resulted in great material loss without significant improvements in purity. Physical properties determined for the isolated and purified metabolite is as follows: ir (KBr disc) cm^{-1} 3400 (broad band OH), 1735, 1645, 1595 and 1075; uv (MeOH) nm (log ϵ) 240 (4.3979), 275 (4.5149), 400 (3.8220) and 440 (3.7708); ^{1}H-nmr (CD_3OD), ppm, 7.24 (s, 1H, 9–H), 5.46 (broad s, 1H, 17–H), 5.07 (d, 7 Hz, 1H, C-1 proton of sugar), 4.65 (s, 1H, 5–H), 3.89 (s, 3H, OCH_3), 3.63 (broad multiplet), 2.79 (s, 2H, 4–H), 2.10 (broad multiplet, 2H, 18–H), 1.52 (s, 3H, 23–CH_3), 1.21 (broad multiplet, 2H, 19–H), 0.93 (s, 3H, 21– or 22–CH_3) and 0.48 (s, 3H, 21– or 22–CH_3); ^{13}C-nmr (d_6-DMSO), see table 1; Field Desorption mass spectral analysis, 767 $(M+1+K)^+$, 751 $(M+1+Na)^+$, 728 $(M+1)^+$ (for $C_{36}H_{41}N_1O_{15}+1$), 715 $(M+1+Na-2\ H_2O)^+$, 695 $(M+1+Na-C_4H_8)^+$, 589 $(M+1+Na-C_6H_{11}O_5)^+$, 565 $(M-C_6H_{11}O_5)^+$, 549 $(M-C_6H_{11}O_5-NH_3)^+$ and 530 $(M-C_6H_{11}O_5-2\ H_2O)$.

HYDROLYSIS OF 2 USING GLUCURONIDASE.—A sample containing 1.5 mg of (2) and 60 mg of β-glucuronidase (Sigma enzyme from *Helix pomatia*) in 20 ml of 0.1 M, pH 5.02 acetate buffer was incubated in a 50 ml Delong flask at 37° for 20 hr. The reaction mixture was then extracted with 10 ml portions of 9:1 ethyl acetate-*n*-butanol until the organic supernatant solutions were colorless. Analytical tlc and uv spectral comparison of the concentrated organic extract revealed that the sole component was viridicatumtoxin. The aqueous layer was frozen and lyophilized, and then extracted with methanol. The resulting methanol extract was passed through 3.5 g of Dowex-50W (H^+-form) (Sigma) previously prepared in methanol. The eluant was concentrated, and its contents were analyzed by paper chromatography and by gc/mass spectral analyses of the pertrimethylsilyl sugar derivative.

CHEMICAL HYDROLYSIS OF METABOLITE 2.—Sulfuric acid (0.4 ml, 1N) was added to 8 mg of 2 in 0.4 ml of methanol, and the contents were refluxed in a 5 ml round bottom flask under nitrogen for 12 hours. After the reactants were cooled to room temperature, 50 mg of barium carbonate was added and allowed to stand at room temperature for another 12 hours. The contents were filtered and the filtrate was extracted thoroughly with 9:1 ethyl acetate-*n*-butanol (9:1) until organic extracts were colorless. Evaporation of the solvent gave a product which was identical to that derived from enzymatic hydrolysis and to standard viridicatumtoxin by tlc, hplc and uv spectroscopy. The aqueous solution remaining was frozen and lyophilized, and the resulting sugar moiety was subjected to paper chromatographic analysis.

RESULTS AND DISCUSSION

Screening experiments with 220 microorganisms, including yeasts, fungi, actinomycetes and other bacteria, were conducted to identify those capable of accumulating metabolites of viridicatumtoxin (1). Many of the cultures examined had been previously used for modification of other polyfunctional antitumor compounds (1,2). Of the organisms examined, 16 accumulated metabolites of viridicatumtoxin; these cultures are listed in table 2. Ten cultures produced a common metabolite, 2, while four others produced a very polar substance with a tlc mobility of Rf 0.1. Two *Streptomyces rimosus* strains produced five or six metabolites of viridicatumtoxin, all in relatively low yield. *Rhizopus* species UI–224 reproducibly formed the major and most common metabolite in the highest overall yields, and this organism was employed to produce sufficient quantities of the metabolite for structure elucidation and biological evaluation. The metabolite was isolated from *Rhizopus* cultures by solvent extraction and was purified by preparative thin layer chromatography. The metabolite was difficult to purify due to apparent instability in solvents and on the usual chromatographic stationary phases.

Unlike viridicatumtoxin itself, the metabolite did not yield usable electron impact mass spectral data. However, the field desorption mass spectrum revealed an apparent molecular ion of *m/e* 728 for $C_{36}H_{41}N_1O_{15}+1$ which indicated the presence of a sugar moiety in the metabolite, while a fragment ion at *m/e* 565 indiciated that the viridicatumtoxin skeleton was most likely intact. Infrared

TABLE 2. Cultures metabolizing viridicatumtoxin.

Culture No.	Culture name	Metabolites	
		2	unknowns
27-UI	*Absidia coerulia* (−)	+	−
7600-UI	*Absidia spinosa* var *biappendiculata*	+	−
811-UI	*Allescheria boydii*	−	+
130-UI	*Corymespora cassicola*	−	
198-UI	*Cunninghamella blakesleeana*	+	−
1369-NRRL	*Cunninghamella blaskesleeana*	+	−
8688a-ATCC	*Cunninghamella blakesleeana*	+	−
9244-ATCC	*Cunninghamella echinulata*	+	−
142-UI	*Cyathus stercoratium*	+	−
804-UI	*Monosporium apiospermum*	−	+
251-UI	*Penicillium chrysogenum*	+	−
70-UI	*Penicillium frequentans*	−	+
224-UI	*Rhizopus species*	+	−
4136-UI	*Stemphylium consortiale*	+	−
2234-NRRL	*Streptomyces rimosus*	−	+
23955-ATCC	*Streptomyces rimosus*	−	+

*All cultures are maintained in the University of Iowa, College of Pharmacy culture collection.

and ultraviolet spectroscopic data were inconclusive for the metabolite, but both proton and carbon-13 nmr spectroscopy were employed in the assignment of the structure of the metabolite as the 11-*O*-β-glucoside (2).

Hydrolysis of the metabolite was accomplished both enzymatically and under acidic conditions to provide aglycone and sugar components for further analysis. It was surprising that glucuronidase from *Helix pomatia* accomplished the ready hydrolysis of the metabolite which yielded viridicatumtoxin itself (verified by tlc and ultraviolet spectroscopy); and glucose (identified by paper chromatography and by gc/mass spectrometric analysis of the pertrimethylsilyl derivative). Neither bovine glucuronidase *beta*-glucosidase nor α-glucosidose sigma, types I and III) were capable of achieving the hydrolysis of the viridicatumtoxin glycoside. While the results of hydrolysis with the enzyme indicated the presence of a glucuronide derivative of viridicatumtoxin, gc/mass spectral and paper chromatographic comparisons of the sugar with glucuronic acid, glucuronic acid lactone and with D-glucose ruled this out. Acidic hydrolysis in sulfuric acid also provided viridicatumtoxin (tlc, hplc, and uv spectral analyses) and the sugar component, which was identified as glucose.

Carbon-13 nmr spectroscopic analyses also supported the presence of a glycosidic residue attached at an unknown point on the viridicatumtoxin skeleton. Assignments of signals in the carbon-13 spectrum were first made for viridicatumtoxin itself, and then for the metabolite. These are summarized in table 1.

The four carbonyl resonances were assigned by analogy with the tetracyclines (8). Aromatic carbons bearing oxygen atoms were assigned by use of the gated spectrum (9) for carbon-8, and signals for carbons 11 and 10 on the basis of spectral differences of viridicatumtoxin in d_6-DMSO and $CDCl_3$:CD_3OD (1:1) solvents (10–11). A chemical shift assignment for position 6a was derived by analogy with chromomycin A_3, (12) and by consideration of the differences in chemical shifts of bridgehead carbon atoms in naphthalene and acenaphthalene. Of the olefinic quaternary carbons, the signal for carbon 16 was assigned by virtue of its long range coupling in the gated spectrum, and signals for carbons at 5a, 6 and 7 were made by analogy with model compounds naphthalene, acenaphthalene, *O*-cresol, *O*-t-butylphenol and chromomycin A_3. Of the three remaining olefinic quaternary resonances, 10a was assigned on the basis of its coupling with a *meta*-

TABLE 1. Carbon-13 nmr spectral assignments for viridicatumtoxin (1) and its metabolite (2).

Carbon	1 d_6-DMSO	1 $CDCl_3$:CD_3OD (1:1)	2 d_6-DMSO
1	194.8	192.8	195.2
2	98.6	98.6	98.7
3	192.3	191.0	192.7
4	—	40.9	—
4a	74.4	71.6	74.5
5	69.4	71.0	70.5
5a	133.6	136.0	132.9
6	127.9	124.6	128.8
6a	147.5	146.9	147.5
7	120.4	122.0	122.5
8	158.8	160.0	158.0
9	99.3	99.2	101.8
10	157.3	157.1	157.3
10a	105.6	105.1	107.4
11	163.3	164.4	162.7
11a	107.3	—	108.4
12	198.8	195.9	199.3
12a	81.4	80.1	81.7
13	173.1	172.4	173.3
14	33.7	33.4	33.8
15	58.8	59.6	58.6
16	137.4	136.5	137.9
17	120.2	120.8	120.3
18	22.6	22.5	22.6
19	—	40.9	—
20	38.2	38.0	38.3
21	25.7*	25.0*	25.8*
22	24.1*	23.5*	24.2*
23	21.0	20.5	21.1
24	55.5	55.0	55.7
1'	—	—	100.5
2'	—	—	73.8
3'	—	—	77.8
4'	—	—	69.5
5'	—	—	77.0
6'	—	—	61.0

*Chemical shift assignment may be interchangeable.

proton, carbon 2 by analogy with tetracyclines, and the signal for 11a by default. Signals for carbons 9 and 17 were made by literature analogy (9).

Of two oxygenated aliphatic quaternary carbon signals, carbon 12a was assigned to that at 81.4 ppm because of its proximity to two electron-withdrawing carbonyl groups, and the higher field 74.4 ppm signal was assigned to carbon 4a. The doublet at 69.4 ppm was assigned to the oxygenated methine carbon at position 5. Signals for carbons 15 and 20 were assigned on the basis of the number of *beta*-substituents (9).

Spectra of viridicatumtoxin (1) in d_6-DMSO revealed signals for only two methylene carbon atoms, while the remaining signals were obscured by solvent peaks. Use of a mixture of $CDCl_3/CD_3OD$ (1:1) revealed an additional overlapping set of signals at 40.9 ppm corresponding to the other two methylene signals. The high field 22.6 ppm signal was assigned to carbon 18 by analogy with di- and tri-terpene examples previously described in the literature. The signal at 33.8 ppm was assigned to the benzylic methylene carbon atom at position 14, which experiences an upfield shift due to gamma interactions. The quartet at 55.5 ppm was assigned to the methoxyl group, and the high field methyl signal at 21 ppm was assigned to carbon 23 due to large $^1J_{CH}$ in SFORD spectra, and to observed long range coupling between it and the vinylic proton in the gated spectrum. The

remaining two methyl group signals at 25.7 ppm and 24.1 ppm are difficult to distinguish from one another and are assigned to the gem-dimethyl group.

The carbon-13 nmr spectrum of the metabolite displayed nearly all of the resonances for carbon signals of viridicatumtoxin along with additional resonances for an attached sugar residue. Under similar experimental conditions, spectral lines of the metabolite were broader than those of viridicatumtoxin itself even following further hplc or other chromatographic purification of the metabolite.

The precise assignment of the point of attachment of the glucose residue to viridicatumtoxin is difficult to achieve by interpretation of ^{13}C-nmr spectra. On the other hand, ^{1}H-nmr provides indirect evidence based on the solvent-induced shift of the proton at position 9. This is the only aromatic proton present in the structures of **1** and **2**. Demarco *et al.*, (14) have clearly shown that protons existing *ortho* to phenolic hydroxyl groups experience a significant shift when ^{1}H-nmr spectra are recorded in different solvents. To verify the existence of such an effect in solvents employed in our work, model compounds *m*-dimethoxybenzene and *m*-methoxyphenol were examined in d_6-DMSO and d_5-pyridine. The proton at position 2 of dimethoxybenzene exists at 6.49 and 6.64 ppm in d_6-DMSO and d_5-pyridine, respectively, (delta of 0.15 ppm), while that for *m*-methoxyphenol occurs at 6.41 and 6.75 in the same solvents (delta of 0.34 ppm). The aromatic proton of viridicatumtoxin (**1**) exhibits resonances at 6.70 ppm in d_6-DMSO and 7.04 ppm in d_5-pyridine, and the shift of this proton signal in the metabolite occurs at 7.39 ppm in d_6-DMSO and 7.90 in d_5-pyridine. These results suggest the presence of a free position-10 hydroxyl group in the structure of the metabolite and support the most likely position for the glycosidic linkage through the 11-hydroxyl group of viridicatumtoxin as in **2**.

Comparison of the carbon-13 chemical shift values of the sugar residue of the metabolite (**2**) with those obtained with model compounds *alpha*- and *beta*-(p-nitrophenyl) glucopyranosides (15), and with those obtained for *beta*-glucosides of lignans such as symplocosin and (+)-epipinoresinol (16) strongly suggest a *beta*-glycosidic linkage for the metabolite.

Microbiological glycosylation reactions have not been widely reported in the literature. Perlman and coworkers reported on the application of a fungal enzyme preparation to achieve transglycosylation reactions with antibiotics (17,18). Glucosidation reactions have been achieved on phenolic anthraquinone substrates, as well (19,20). Kieslich and coworkers described the formation of various 4-O-methylglucosides and glucosides with phenolic substrates by *Sporotrichum* and *Rhizopus* species (21,22), and Otten and Rosazza described the formation of the β-glucoside of the naphthoquinone lapachol (23).

Glycosylation reactions may be of some value in preparing more solubilized derivatives of antitumor compounds such as viridicatumtoxin and other phenolic agents. This philosophy was unsuccessfully exploited in studies with the anthracycline aglycone, daunomycinone (24). Viridicatumtoxin-11-*O*-β-glucoside (**2**) was examined for antitumor activity vs. the P-388 leukemia test system (25), and was found to be totally inactive.

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