Usnic Acid Derivatives as Potential Antineoplastic Agents

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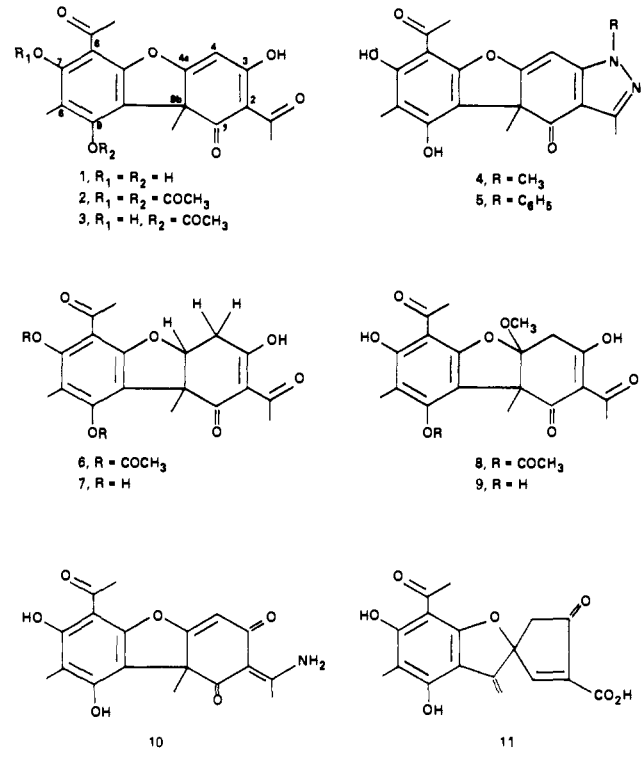
Usnic acid, a lichen antibiotic, showed low-level activity in the Lewis lung carcinoma test system. In an effort to produce new agents of potential use in the treatment of lung cancer, derivatives of the natural product were synthesized and evaluated with a cytotoxicity assay. Structure–activity analysis of the cytotoxicity data indicated the importance of the lipophilicity and the β-triketone moiety of usnic acid on cytotoxicity. No significant increases in survival of test animals over controls were shown by any of the synthetic compounds in the P388 leukemia or the Lewis lung carcinoma test systems.

In contrast to developing agents of nonspecific cytotoxicity, there is a need to develop antitumor drugs that are disease specific (e.g., neuroblastoma and melanoma) or organ specific (e.g., pancreas, brain, and lung). Chemotherapy, either alone or as part of a combined modality regimen, is indicated in close to 90% of all patients with lung cancer.[2] Because lung cancer among all neoplastic diseases annually claims the most fatalities in industrialized countries, there clearly is a need to develop antitumor agents with lung specificity. Responsive to the clinical need, we set out to generate some leads, and from those synthesize compounds which might have some potential in the treatment of lung cancer. With computer assistance, the entire National Cancer Institute biological file (ca. 300 000 compounds) was searched to identify all those compounds which had shown confirmed activity in the Lewis lung carcinoma test system. Including clinically well-known folic acid antagonists, mitosis inhibitors, and antimetabolites, less than 100 compounds fit our predetermined criteria for activity ($T/C \geq 125$) and reproducibility ($T/C \geq 125$ in two or more tests). Members of the mustard and nitrosourea classes are decidedly the most efficacious against the Lewis lung tumor. However, the large numbers of mustards and nitrosoureas that have been synthesized and evaluated for antitumor properties have not provided a drug with good clinical activity against lung cancer. Since we had committed ourselves to the synthesis of lung-specific antitumor agents, it was reasonable to consider as possible lead compounds the more novel, less familiar structures resulting from the computer search. Usnic acid, an antibiotic which has been isolated from species of several lichen genera, was among the more novel structures having confirmed, albeit modest, Lewis lung activity.

The inhibition of Lewis lung carcinoma by (−)-usnic acid in a gum acacia suspension has been reported by Kupchan and Kopperman,[3] who isolated the antibiotic from a *Cladonia* species. Within a dose range of 20–200 mg/kg, the life span of tumored mice was increased 35 to 52% over the untreated control group. This moderate antitumor activity prompted us to consider the synthesis of some (−)-usnic acid derivatives with the hope of finding one with greater activity in the Lewis lung system.

Of the β-triketones isolated from natural sources, usnic acid (1), in both the *d* and *l* modifications, is ubiquitous.[4] In spite of its high percentage composition of oxygen, the solubility of 1 in water (<10 mg/100 mL, 25 °C) and ethanol (20 mg/100 mL, 25 °C) is surprisingly low.[5] These observations, in conjunction with the observed good chloroform solubility, were interpreted[6] as arising from three strong intramolecular hydrogen bonds. Since a



QSAR study[7] of nitrosoureas vs. the Lewis lung tumor revealed an ideal log *P* value of approximately 1, poor activity against Lewis lung could be associated with large deviations from this ideal log *P*. The log *P* of (−)-usnic acid in the octanol/water system was found to be 2.88 by direct measurement. Apparently, the intramolecular hydrogen bonds not only contribute to the poor solubility in hydroxylic solvents but also lead to a log *P* value which is well into the lipophilic range.

In exploring the antitumor potential of usnic acid congeners, it seemed reasonable to prepare and test simple derivatives wherein some of the intramolecular hydrogen bonds are disrupted, resulting in a decreased lipophilicity. In addition to exploring the effect of log *P* on the biological activity of 1, derivatives were also selected for synthesis with the intent of assessing the role of the β-triketone function on activity.

Chemistry. Compounds 1–11 were prepared according to literature procedures or by standard methods. References for the literature procedures are given in Table I, along with percentage yields, melting points, and specific rotations. Although there are a number of possible structures for the compound isolated from the reaction of

Table I

no.	yield, % (proced ref)	mp, °C	lit. mp (ref)	$[\alpha]_D$, deg (concn)	lit. $[\alpha]_D$, deg (ref)[f]	log P[g]
2	60 (8)	201-202	203-204 (9)	-233 (0.64)	-235 (9)	2.5
3	83 (10)	157-158[a]		-294 (0.64)		2.7
4	55	205-206		-518 (0.17)		2.8
5	34 (11)	194-195	198-199 (9)	-660 (0.60)	-604 (9)	3.2
6	86[b] (13)	147-148	148-149 (9)	-7 (2.3)	-6 (9)	2.3
7	67 (12)	144-145	147-148 (9)	+94 (1.85)	+83 (9)	2.4
8	80[c] (13)	165-166	167-168 (13)	+74 (2.8)	+68.8 (13)	2.6
9	60[d] (13)	97 dec	92 dec[e] (13)	+32 (0.65)	-44.2[e] (13)	2.3
10	88 (14)	249-250	252-254 (14)	-424 (0.08)	-230 (14)	3.2
11	40 (15)	230 dec	230 dec (15)			1.3

[a] The 9-*O*-acetate prepared[10] from racemic usnic acid had mp 190-191 °C. [b] The NMR spectrum showed the product to be a mixture (2:1) of the two C-4a isomers. [c] As reported[16] the 4a-α- and 4a-β-methoxy isomers are clearly shown in a composite NMR spectrum. [d] The NMR spectrum indicated a mixture of the 4a-α- and 4a-β-methoxy isomers. [e] The melting point (or specific rotation) is that observed when (+)-usnic acid is used as starting material. [f] Specific rotations were measured in chloroform solutions; concentrations are expressed in g/100 mL of solvent. [g] Partition coefficients were determined by high-performance LC using the method of McCall[17] with a reverse-phase octadecylsilyl column eluted with acetonitrile-water (60:40). Usnic acid, which has an experimental determined log *P* of 2.88, was used to calibrate the system.

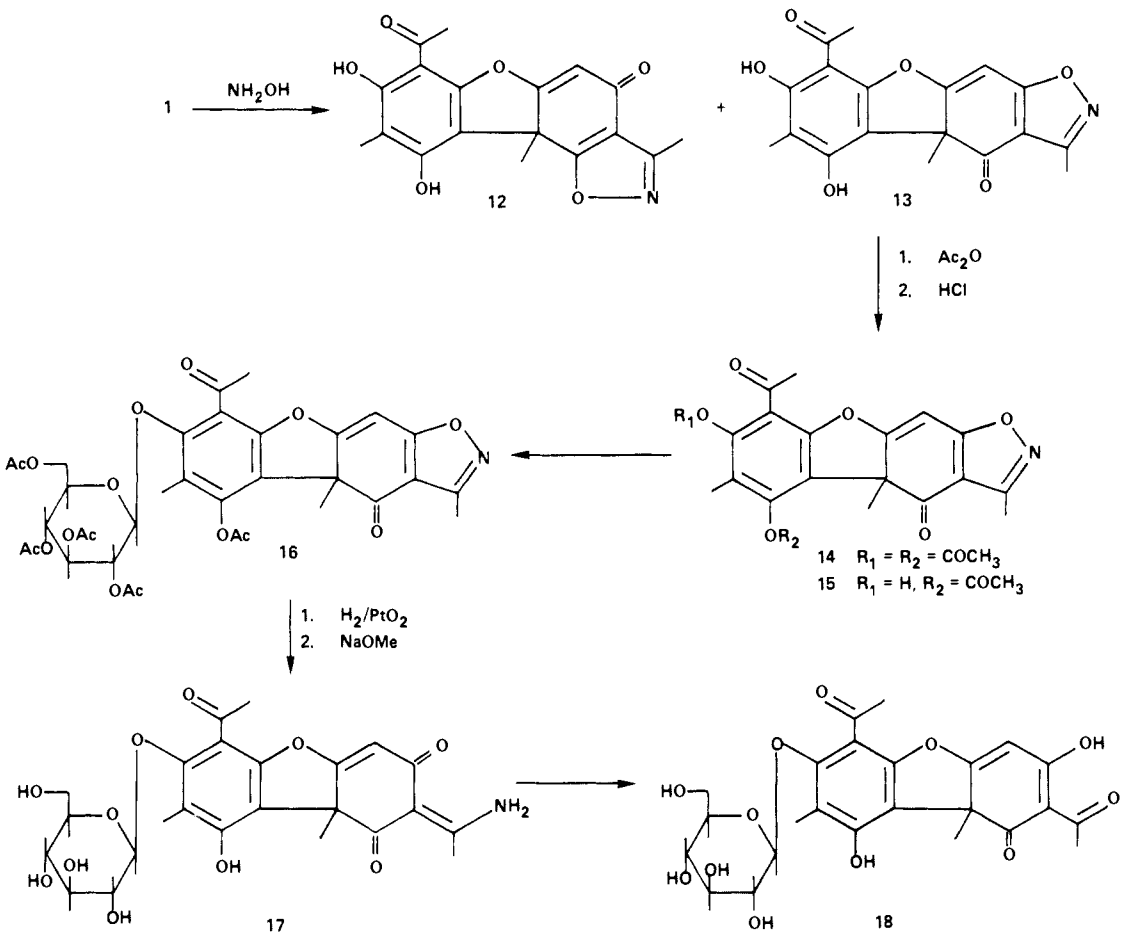
methylhydrazine with **1**, structure **4** was assigned to the product in order to be consistent with the structure of the product obtained with phenylhydrazine (**5**), which was confirmed[9] by chemical degradation. In line with expectations, both **4** and **5** had similar UV and NMR spectra.

The monoacetate **3** was formed by selective hydrolysis of the diacetate with aqueous sodium carbonate. The location of the remaining acetyl group follows[10] from the NMR spectrum of **3**, which lacks the phenolic proton at δ 11.07 which appears in the spectrum of **1**. The proton of the other phenolic hydroxyl group, located at the 7 position of **1**, characteristically appears further downfield at δ 13.28, indicative of a stronger intramolecular hydrogen bond than that formed by the 9-position phenolic hydrogen. Accordingly, the monoacetate **3** shows a singlet at δ 13.21 in agreement with the assigned structure. Since the formation of the monoacetate **3** necessitates destruction of an intramolecular hydrogen bond concomitant to the addition of the hydrophilic acetyl group (π = -0.55),[18] it was hoped that **3** would have a lower log *P* relative to **1** than the 0.4 decrease actually observed. In general, the compounds in Table I cover a rather narrow log *P* range, which lies quite far into the lipophilic region. Although usnolic acid (**11**) has a log *P* in what might be considered a more optimal range, it perhaps cannot be considered a true analogue of **1** because of the large structural differences contrasting them. In order to better explore the role of log *P* on usnic acid congeners, it occurred to us that substitution of a glucosyl moiety (π = -2.84)[18] on a phenolic hydroxyl of **1** would effectively offset the lipophilic character of **1** without requiring major structural alterations. Thus, synthesis of glucosides derived from usnic acid would permit the biological study of this series of compounds in a significantly different log *P* range. The glucosyl carrier can be considered of the disposable variety because in vivo action of a glucosidase could liberate the aglycon.

In order to successfully condense a glucose intermediate with **1**, it was obligatory to mask the reactive β-triketone function. The blocking method of Kutney and coworkers[19] seemed ideally suited to our needs. Accordingly, a suspension of (-)-usnic acid (**1**) in ethanol/pyridine was reacted with hydroxylamine hydrochloride[20] (Scheme I) to give a mixture of **12** (8%) and **13** (51%), which was separable by chromatography. By analogy to the usnic acid monoacetate preparation, the diacetate of **13** was selectively hydrolyzed to the monoacetate **15**. In agreement with the assigned structure, the NMR spectrum of **15** showed the three protons of the C-9 acetoxy group at δ 2.74 and the C-7 phenolic proton at δ 13.21. Koenigs–Knorr condensation of the monoacetate **15** with tetra-*O*-acetyl-α-D-glucopyranosyl bromide[21] in the presence of silver oxide gave **16** as a yellow amorphous powder. Since glycosidation using the Koenigs–Knorr synthesis normally results in the formation of the β anomer,[22] compound **16** can be assigned the β configuration. Reductive cleavage of the isoxazole ring of **16** with hydrogen afforded a vinylogous imide, which was treated with sodium methoxide in methanol to effect removal of the acetyl blocking groups. The resulting enamino usnic acid glucoside **17** on mild treatment with 1.2 N sodium hydroxide gave **18**. Since the anomeric proton of the sugar moiety of **18** appeared as a doublet at δ 4.47 ($J_{1',2'}$ = 7 Hz), indicating a trans-diaxial relationship to the vicinal 2′ proton, the β configuration at the glycosidic bond was, therefore, further supported.[23] Both glucoside derivatives (**17** and **18**) had good water solubilities, as anticipated. By experimental determination, the partition coefficient (log *P*) of **18** was found to be 0.56, which is within a range that would allow us to probe the effect of log *P* on antitumor activity in the usnic acid series. The octanol–water partition coefficients measured for **1** and **18** enabled a direct determination of -2.99 for the π constant of the *O*-β-glucopyranosyl substituent. This is in good agreement with the literature[18] value of -2.84, which was calculated from the log *P* of phenyl β-D-glucopyranoside.

Biological Results and Discussion. The usnic acid derivatives were evaluated in a cytotoxicity assay, the results of which are shown graphically in Figure 1. Exponentially growing L1210 cells were exposed to each compound in parallel experiments at a concentration of 1.4×10^{-7} mol/mL, and growth inhibitions were determined after 23 and 46 h of incubation. The compounds clustered into three groups in terms of the extent to which they inhibited the growth of the cultured cells. The first group, including usnic acid, its acetates, and close analogues (**1–3** and **7–10**), almost completely inhibited cell growth at 1.4×10^{-7} mol/mL. The second group (**4–6** and **12**), having a somewhat lesser cytotoxicity, are derivatives wherein modifications to the β-triketone functionality were made. Although the intact β-triketone portion of the molecule is apparently important for activity, one might have anticipated an even greater than observed reduction in cytotoxicities resulting from chemical alterations at the β-triketone. Other indications of cytotoxicity associated with the β-tricarbonyl moiety have been noted among

Scheme I



compounds submitted to the NCI for antitumor testing.[24] For example, the mold metabolite, citrinin,[25] showed reproducible activity against murine P388 leukemia.

With the exception of **13**, which precipitated from the culture medium, the more hydrophilic compounds in this series (**11**, **17**, and **18**) constitute the third, and least cytotoxic, group which can be recognized in Figure 1.

Work with cultured L1210 cells identified the more lipophilic derivatives in the usnic acid series as the most cytotoxic and indicated that the β-triketone function was necessary for the maximum obtainable cytotoxicity in this series. Unfortunately, none of the synthetic derivatives appeared to be more potent than the parent compound. Nevertheless, it was our opinion that a complete evaluation of these compounds could only be achieved through the use of relevant in vivo antitumor assays. In addition to activity against the Lewis lung tumor, usnic acid (**1**) also showed some activity in the murine P388 leukemia test system (T/C = 141 at 100 mg/kg). The P388 activity was potentially useful for this study because the P388 system gives more accurate T/C values resulting from a narrower control–animal death range than is observed for the biologically more complicated Lewis lung system. Therefore, all compounds were tested in duplicate against P388 leukemia[26] and Lewis lung carcinoma.[27] Compounds were administered according to a chronic schedule in gum acacia suspensions. The results recorded in Table II show some sporadic active indications which generally could not be reproduced. The complete lack of activity in the P388 system shown by the water-soluble glucoside derivatives **17** and **18** was particularly disappointing. However, the synthesis of additional derivatives having partition coefficients greater than that of usnic acid (**1**) seemed academic at best because formulation problems to be anticipated with very lipophilic drugs would preclude any ultimate clinical application.

Experimental Section

Routine purity analyses and the determination of relative partition coefficients were carried out with a Waters Associates, Inc., Model ALC/GPC-244 liquid chromatograph equipped with a 4 (i.d.) × 300 mm μBondapak C_{18} column. Mass spectra were obtained by direct probe insertion with a DuPont 21-492 spectrometer using a 75-eV ionizing voltage. When necessary, samples were derivatized for MS as previously described.[28] Proton NMR spectra were recorded with a Varian HA-100D spectrometer. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane, which was used as an internal standard. A Cary Model 15 spectrophotometer was used to obtain UV spectra and a Perkin-Elmer Model 621 was used to record infrared spectra. Specific rotations were measured with a Perkin-Elmer Model 141 polarimeter at the sodium D line using a 1-dm cell. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by the Section on Microanalytic Services and Instrumentation, NIAMDD, NIH, and by Galbraith Laboratories, Inc., Knoxville, Tenn. The analytical results for the elements indicated by their symbols were within ±0.3% of theoretical values. Supplies of (−)-usnic acid were purchased from ICN Pharmaceuticals, Inc., Cleveland, Ohio [lot no. 1694, from *Ramalina reticulata*, $[\alpha]_D$ −560° (*c* 0.47)] and from Sigma Chemical Co., St. Louis, Mo. [lot no. 96C-0234, from *Cladonia alpestris*, $[\alpha]_D$ −530° (*c* 0.36)].

8-Acetyl-1,4a-dihydro-5,7-dihydroxy-1,3,4a,6-tetramethyl-4*H*-benzofuro[3,2-*f*]indazol-4-one (**4**). A suspension of (−)-usnic acid (3.95 g, 11.5 mmol) in ethanol (40 mL) was treated with methylhydrazine (0.6 g, 13 mmol) and refluxed for 2 h. After

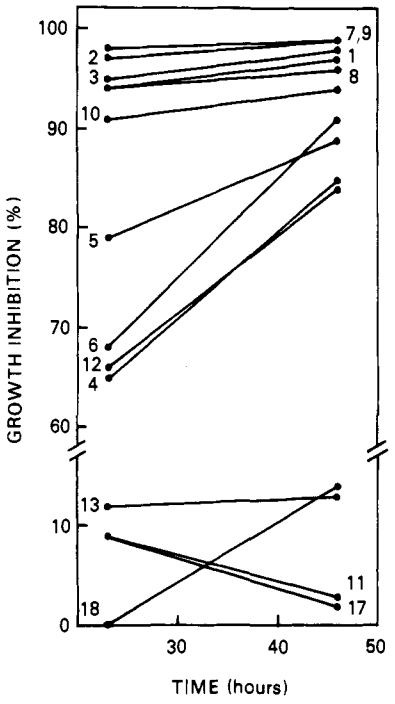


Figure 1. Cytotoxicity evaluations. Cultured L1210 cells were continuously exposed to compound at ca. 1.4×10^{-7} mol/mL. Cell counts were made at the end of 23 and 46 h. The percent growth inhibition with time of a treated population with respect to a control is shown for the usnic acid derivatives, each of which is identified by a compound number. Compound 13 precipitated from solution.

evaporation of the solvent, the residue was crystallized from ethanol to give 2.25 g of pale-yellow granules: UV (CH_3OH) λ_{max} 227 nm (ϵ 41 700), 246 (27 300), 285 (20 200); IR ($CHCl_3$) 1682, 1630 cm^{-1}; NMR ($CDCl_3$) δ 1.69 (s, 3 H, C_{9b}-CH_3), 2.07 (s, 3 H, C_8-CH_3), 2.47 (s, 3 H, C_{11}-CH_3), 2.65 (s, 3 H, C_6-$COCH_3$), 3.83 (s, 3 H, N-CH_3), 6.11 (s, 1 H, C_4-H), 11.14 (s, 1 H, C_9-OH), 13.27 (s, 1 H, C_7-OH). Anal. ($C_{19}H_{18}O_5N_2$) C, H, N.

8-Acetyl-5,7-dihydroxy-3,4a,6-trimethylbenzofuro[3,2-*f*]-1,2-benzisoxazol-4(4a*H*)-one (13). Using a literature procedure,[20] 15.0 g of (−)-usnic acid was converted to 7.6 g (51%) of 13 after silica gel chromatography and crystallization from EtOAc: mp 223–224 °C dec [lit.[20] mp 230–231 °C, (+) isomer]; $[\alpha]_D$ −680° (*c* 0.26, $CHCl_3$) [lit.[20] $[\alpha]_D$ +669°, (+) isomer].

7-Acetyl-8,10-dihydroxy-3,9,10b-trimethylbenzofuro-[2,3-*g*]-1,2-benzisoxazol-4(10b*H*)-one (12). The chromatography of the foregoing reaction product, from the reaction of (−)-usnic acid with hydroxylamine, also gave 1.2 g (8%) of 12 after crystallization from EtOAc: mp 183–184 °C dec (lit.[20] mp 178–179 °C, (+) isomer); $[\alpha]_D$ −825° (*c* 0.24, $CHCl_3$) (lit.[20] $[\alpha]_D$ +851°, (+) isomer).

8-Acetyl-5,7-bis(acetyloxy)-3,4a,6-trimethylbenzofuro-[3,2-*f*]-1,2-benzisoxazol-4(4a*H*)-one (14). A solution of 13 (23.6 g, 69.1 mmol) in 240 mL of acetic anhydride containing 0.5% concentrated sulfuric acid was maintained overnight at 60 °C. The reaction mixture was poured into ice–water (2 L) with stirring. The precipitate was collected and crystallized from EtOH to afford 20.5 g (70%) of yellow needles: mp 179.5–180 °C; $[\alpha]_D$ −428° (*c* 0.114, $CHCl_3$); UV (CH_3OH) λ_{max} 247 nm (ϵ 14 600), 305 (5450), 357 (3470); IR ($CHCl_3$) 1771, 1698, 1664, 1612, 1558 cm^{-1}; NMR ($CDCl_3$) δ 1.83 (s, 3 H, C_{9b}-CH_3), 1.99 (s, 3 H, C_8-CH_3), 2.61 (s, 3 H, C_{11}-CH_3), 2.52 (s, 3 H, C_6-$COCH_3$), 2.46 (s, 3 H, C_9-$OCOCH_3$), 2.32 (s, 3 H, C_7-$OCOCH_3$), 6.32 (s, 1 H, C_4-H); MS *m/e* 425 (M^+). Anal. ($C_{22}H_{19}O_8N$) C, H, N.

8-Acetyl-5-(acetyloxy)-7-hydroxy-3,4a,6-trimethyl-benzofuro[3,2-*f*]-1,2-benzisoxazol-4(4a*H*)-one (15). The diacetate 14 (20.5 g, 48.2 mmol) was dissolved in hot methanol (1 L) and treated with 1 L of 6 N HCl solution. The solution was refluxed for 0.5 h and cooled to room temperature, and the precipitate was collected by filtration. The product was crystallized from MeOH to give 12.9 g (70%) of yellow needles: mp 175–176 °C; $[\alpha]_D$ −482° (*c* 0.087, $CHCl_3$); UV (CH_3OH) λ_{max} 250 nm (ϵ 13 400), 265 (12 000), 357 (5740); IR ($CHCl_3$) 1776, 1696, 1637, 1612, 1562 cm^{-1}; NMR ($CDCl_3$) δ 1.79 (s, 3 H, C_{9b}-CH_3), 2.02 (s, 3 H, C_8-CH_3), 2.46 (s, 3 H, C_{11}-CH_3), 2.51 (s, 3 H, C_6-$COCH_3$), 2.74 (s, 3 H, C_9-$OCOCH_3$), 6.32 (s, 1 H, C_4-H), 13.21 (s, 1 H, C_7-OH); MS *m/e* 383 (M^+). Anal. ($C_{20}H_{17}O_7N$) C, H, N.

8-Acetyl-5-(acetyloxy)-3,4a,6-trimethyl-7-[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)oxy]benzofuro[3,2-*f*]-1,2-benzisoxazol-4(4a*H*)-one (16). The monoacetyl derivative 15 (15.0 g, 39.1 mmol) was combined with 19.2 g (46.7 mmol) of tetra-*O*-acetyl-α-D-glucopyranosyl bromide[21] in anhydrous acetonitrile (200 mL), treated with 10.8 g of silver(I) oxide, and refluxed for 2.5 h. The reaction mixture was cooled to room temperature and the inorganic material removed by filtration through Celite. The filter cake was washed several times with $CHCl_3$, and the filtrate and washings were evaporated. The residue in benzene solution was applied to a silica gel column. Elution with benzene–acetone (20:1) returned 5.0 g (33%) of starting material (15). Continued elution gave 5.9 g (21%) of 16, which was deposited as a yellow powder from methanol: mp 135 °C dec; $[\alpha]_D$ −288° (*c* 0.09, $CHCl_3$); UV (CH_3OH) λ_{max} 218 nm (ϵ 30 000), 247 (13 600), 297 (6360), 358 (3390); IR ($CHCl_3$) 1763, 1694, 1657, 1603, 1560 cm^{-1}; NMR ($CDCl_3$) δ 1.81 (s, 3 H, C_{9b}-CH_3), 2.07 (s, 3 H, C_{11}-CH_3), 2.03 (s, 3 H, C_8-CH_3), 2.08 (s, 3 H, C_6-$COCH_3$), 2.10 (s, 3 H, C_9-$OCOCH_3$), 2.12, 2.46, 2.53, 2.55 [4 s, 4 × 3 H, sugar $(OCOCH_3)_4$]. Anal. ($C_{34}H_{35}O_{16}N$) C, H, N.

6-Acetyl-2-(1-aminoethylidene)-7-(β-D-glucopyranosyl-

Table II

	in vivo antitumor evaluations[a]	
no.	P388 leukemia[b] T/C (dose, mg/kg)	Lewis lung carcinoma[c] T/C (dose, mg/kg)
2	115 (6)	117 (12)
	144 (200)	111 (6)
3	119 (200)	112 (50)
	113 (200)	118 (25)
4	119 (50)	112 (200)
	118 (50)	103 (12)
5	110 (200)	111 (25)
	122 (6)	108 (100)
6	112 (50)	114 (50)
	108 (25)	104 (3)
7	118 (25)	107 (50)
	128 (100)	107 (12)
8	124 (100)	108 (50)
	106 (25)	
9	128 (25)	122 (25)
	122 (50)	106 (50)
10	112 (6)	111 (50)
	107 (6)	110 (6)
11	112 (12)	127 (50)
	108 (25)	109 (100)
12	114 (100)	117 (12)
	120 (100)	112 (6)
13	104 (100)	111 (50)
	104 (100)	
17	108 (25)	
	104 (12)	
18	108 (50)	
	102 (25)	

[a] Dose-response assays were conducted at dose levels of 200, 100, 50, 25, 12, and 6 (mg/kg)/day. Test compounds in gum acacia suspension were administered intraperitoneally to tumored mice on days 1–9 (nine injections). Assays were generally determined in duplicate. The highest T/C values for each assay, along with associated doses, are recorded in the table. A T/C value of ≥125 is considered significantly active, where T/C represents the ratio of the median survival time of the treated animals over those of the control animals expressed as a percentage. [b] Reference 26. [c] Reference 27.

oxy)-9-hydroxy-8,9b-dimethyl-1,3(2*H*,9b*H*)-dibenzofurandione (17). The oxime anhydride derivative 16 (10.7 g, 15 mmol) was hydrogenated in ethanol solution (200 mL), catalyzed by 1.07 g of PtO_2. After the uptake of 1 mol equiv of hydrogen, the shaking was discontinued, and the reaction mixture was filtered through a pad of Celite.

After evaporation of the filtrate under reduced pressure, the residue in 100 mL of methanol was combined with 100 mL of 1 N NaOMe in methanol and stirred at room temperature for 3 h. The reaction solution was then stirred for 3 h with excess Dowex 50-X8 (hydrogen form; washed with MeOH prior to use) to effect neutralization. The resin was removed by filtration and washed with methanol, and the filtrate and washings were evaporated under vacuum. The residue was passed through a DEAE-cellulose column with methanol, and the eluent was evaporated and chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (85:14:1). The middle fractions were combined, evaporated, and dissolved in water. Lyophilization gave 17 (6.55 g, 86%) as a colorless powder: mp 160 °C dec; $[\alpha]_D$ −117° (*c* 0.165, MeOH); UV (MeOH) λ_{max} 224 nm (ϵ 20900), 284 (20100); IR (KBr) 1691, 1603, 1562 cm^{-1}; NMR (D_2O exchanged, CD_3OD) δ 1.66 (s, 3 H, C_{9b}-CH_3), 2.21 (s, 3 H, C_8-CH_3), 2.59 (s, 6 H, C_{11}-CH_3 and C_6-$COCH_3$), 4.49 (d, J = 7 Hz, 1 H, anomeric proton), 5.59 (s, 1 H, C_4-H); MS *m/e* 937 [M^+·(Me_3Si)$_6$]. Anal. ($C_{24}H_{27}O_{11}N$·H_2O) C, H, N, O, H_2O (Karl Fischer).

2,6-Diacetyl-7-(β-D-glucopyranosyloxy)-3,9-dihydroxy-8,9b-dimethyl-1(9b*H*)-dibenzofuranone (18). A solution of the glucoside of enaminousnic acid 17 (4.0 g, 7.6 mmol) in 1.2 N aqueous sodium hydroxide solution (400 mL) was stirred at room temperature for 3 h. Dowex 50-X8 (hydrogen form) resin was added in excess and the stirring continued for an additional 3 h. The resin was removed by filtration and washed with methanol, and the filtrate and washings were combined and evaporated under vacuum (35 °C water bath). The residue was dissolved in a small amount of methanol and the insoluble usnic acid (1), which had formed by hydrolysis, was removed by filtration. The filtrate was run through a cellulose column to remove the glucose which formed concurrently with 1. Final purification of the product from the cellulose column after solvent evaporation was achieved with silicic acid chromatography with $CHCl_3$–MeOH–H_2O (85:14:1). After pooling like fractions and solvent evaporation, the glucoside 18 was obtained as a monohydrate through lyophilization of a water solution. The product (1.6 g, 40%) appeared as a pale-yellow powder: mp 145 °C dec; $[\alpha]_D$ −398° (*c* 0.137, CH_3OH); UV (CH_3OH) λ_{max} 269 nm (ϵ 25500); IR (KBr) 1675, 1605, 1540 cm^{-1}; NMR (D_2O exchanged, in CD_3OD) δ 1.73 (s, 3 H, C_{9b}-CH_3), 2.24 (s, 3 H, C_8-CH_3), 2.57 (s, 6 H, C_{11}-CH_3 and C_6-$COCH_3$), 4.47 (d, J = 7 Hz, 1 H, anomeric proton), 5.85 (s, 1 H, C_4-H); NMR ($CDCl_3$) δ 1.76 (s, 3 H, C_{9b}-CH_3), 2.13 (s, 3 H, C_8-CH_3), 2.47 (s, 3 H, C_{11}-CH_3), 2.60 (s, 3 H, C_6-$COCH_3$), 5.87 (s, 1 H, C_4-H), 10.78 (s, 1 H, C_9-OH), 18.72 (s, 1 H, C_3-OH); MS *m/e* 938 [M^+·(Me_3Si)$_6$]. Anal. ($C_{24}H_{26}O_{12}$·H_2O) C, H, O, H_2O (Karl Fischer).

Cytotoxicity Assay. L1210 Cell Culture. L1210 cells were grown in Roswell Park Memorial Institute Medium 1630 supplemented with 16.7% heat-inactivated fetal calf serum (Flow Laboratories) and gentamicin (Schering) at 40 μg/mL. Exponentially growing cells were harvested (8–10 × 10^5 cells/mL) and washed twice in fresh medium. Growth-inhibition studies were performed in Corning tissue culture flasks (25 cm^2) with growth medium (10 mL) seeded with 1 × 10^5 cells/mL. The drugs in Me_2SO solution were added (15 μL) to the culture flasks to give a final concentration of 1.39 ± 0.17 × 10^{-7} mol/mL. After 23 and 46 h of incubation at 37 °C, cell counts were determined with a Coulter Counter. The effect of each drug on cell proliferation with respect to an untreated control population is presented in Figure 1 as a percentage inhibition observed at 23 and 46 h. Untreated control cells, in the specified medium, grew exponentially with a doubling time of about 12 h.

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References and Notes

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