THE CHEMISTRY OF THE ANTIBIOTICS CHRYSOMYCIN A AND B

ANTITUMOR ACTIVITY OF CHRYSOMYCIN A

U. WEISS* and K. YOSHIHIRA**[a]

Laboratory of Chemical Physics, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health
Bethesda, Maryland 20205, U.S.A.

R. J. HIGHET

Laboratory of Chemistry, National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, Maryland 20205, U.S.A.

R. J. WHITE**[b] and T. T. WEI

Fermentation Program, Frederick Cancer Research Center
Frederick, Maryland 21701, U.S.A.

(Received for publication March 8, 1982)

The yellow antibiotic chrysomycin, isolated in crystalline form in 1955, is found to consist of two closely related components, a major one, chrysomycin A, and a minor one, chrysomycin B. They differ only through the replacement of a vinyl group of chrysomycin A by methyl in chrysomycin B. The absorption spectrum of chrysomycin A is identical with that of the antitumor antibiotic toromycin (gilvocarcin V, 2064A), while that of chrysomycin B is similarly identical with the one of gilvocarcin M (2064B). The structures of these antibiotics (toromycin, the gilvocarcins, and 2064A and B) have been elucidated recently. Chrysomycins A and B thus contain the same chromophores as gilvocarcins V and M, respectively; comparison of ^{1}H and ^{13}C NMR spectra confirms this identity. The chrysomycins differ from these other antibiotics in the *C*-glycosidic side-chain, which is a methylpentose in the gilvocarcins, a 3,5-dimethylpentose in the chrysomycins. Structure and relative configuration of the latter are given. The biological activity and possible biosynthesis of the chrysomycins are discussed.

The yellow, crystalline antibiotic chrysomycin was discovered in 1955 at the New York Botanical Garden by STRELITZ *et al.*[1] during their studies of antibiotic substances active against bacteriophage; these studies had been undertaken in the hope that the antiphage activity might serve as a guide for action against viruses, particularly the poliomyelitis virus.[2] Some physical, chemical, and biological properties of chrysomycin were recorded by STRELITZ *et al.*[1], including the highly characteristic electronic absorption spectrum. However, elementary analyses for C, H, and O did not lead to a consistent empirical formula for the N-free antibiotic.[1] This, and the fact that the ratio of the extinction coefficients of the two major UV peaks varied from one sample to the other,[1] suggests that the chrysomycin of these authors, although nicely crystalline, was not a homogeneous compound. The remarkably low toxicity on intraperitoneal application in mice, noted by STRELITZ *et al.*, was subsequently confirmed by us.

* I wish to dedicate this paper to the memory of my late friend Dr. FRIEDA STRELITZ, New York Botanical Garden, whose tireless effort was mainly responsible for the isolation of chrysomycin and for the accumulation of the material which enabled the present study to be carried out. She passed away, a victim of cancer, in the Spring of 1957.

** Present addresses: (a) National Institute of Hygienic Sciences, Tokyo 158, Japan; (b) American Cyanamid Company, Medical Research Division, Lederle Laboratories, Pearl River, New York 10965, U.S.A.

No further research on chrysomycin has been published since 1955.

Through prodigious effort, Drs. STRELITZ and FLON succeeded in accumulating an appreciable amount of chrysomycin, which served as the material for the work here reported. No additional supplies are obtainable at present, since all the remaining cultures of *Streptomyces* A-19, the original source of chrysomycin, appear to have lost the ability to produce this antibiotic.

Acetylation of chrysomycin led to a product separable by chromatography into the triacetyl derivatives of two distinct, closely related antibiotics, a major component, chrysomycin A (**1**), and a minor one, chrysomycin B (**2**). Attempts to recover the initial antibiotics from their acetates by saponification led to complex mixtures. ^{1}H and ^{13}C NMR spectroscopy of the acetates showed that the structures of **1** and **2** are identical except for replacement of a vinyl group of **1** by a methyl in **2***. Chrysomycin A crystallizes in intergrowths of thin, curved needles under all conditions tried; numerous attempts to grow crystals of the antibiotic (or its triacetate) suitable for X-ray crystallography were uniformly unsuccessful.

Additional interest in the chrysomycins, **1** and **2**, was generated by the work[3] on antibiotics 2064A (**3**) and 2064B (**4**), which were found to be highly active in the biochemical induction assay (BIA) screening test[4] for antitumor activity; this test is based on prophage induction. The 2064 antibiotics showed electronic absorption spectra very similar to that of chrysomycin. Like these antibiotics, chrysomycin proved active in the BIA assay.

Analysis by HPLC led to the ready separation, on an analytical scale, of non-acetylated **1** and **2**, which were found to have electronic spectra completely identical with those of **3** and **4**, respectively, but to differ from them in chromatographic behavior. The same technique also proved that one of the old samples of chrysomycin was almost pure **1** (~98%). High-resolution mass spectrometry and elementary analysis of this sample established the elementary formula $C_{28}H_{28}O_9$ for **1**. In the IR spectrum, a carbonyl band at 1710 cm^{-1} is prominent. The ^{1}H NMR spectrum showed the presence of one pair each of *ortho*- and *meta*-oriented aromatic protons, one isolated aromatic proton, two different aromatic methoxyls, the vinyl group, one secondary and one tertiary non-aromatic *C*-methyl, several hydroxyls, and four non-aromatic protons. The ^{13}C NMR spectrum of **1** showed that the prominent IR band at 1710 cm^{-1} most probably is caused by a coumarin group.

Investigation of the minute amounts of chrysomycin B (**2**) available from the analytical HPLC showed completely analogous behavior. The mass-spectrometric fragmentation of **2** produced the same pattern as that of **1** but with mass values lower by 12, as expected from the replacement of vinyl by methyl. Chrysomycin B is thus $C_{27}H_{28}O_9$; this conclusion was confirmed by the high-resolution mass-measurement of the molecular peak of **2** present as contaminant in a sample of **1**. The fragmentation patterns of **3** and **4**, in turn, were analogous to those of **1** and **2**, but produced ions of mass lower by 14; the empirical formulae of **3** and **4** are therefore $C_{27}H_{26}O_9$ and $C_{26}H_{26}O_9$, respectively. In view of the identity of the electronic spectra of the two pairs (**1** and **3**, **2** and **4**), the structural difference must reside in the non-chromophoric parts of those molecules.

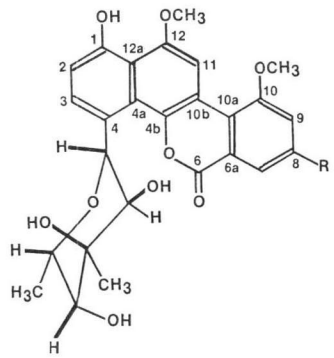
Because of the impossibility of securing additional supplies of the chrysomycins, it was decided to reserve the limited quantity of available material for further biological tests; more detailed chemical studies were thus not feasible. Recently, however, **3** and **4** were found to be identical, spectroscopically

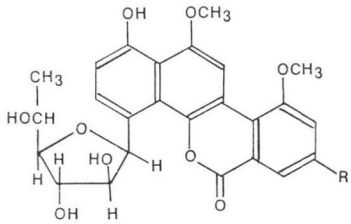
* This relationship between **1** and **2**, while uncommon, is not unprecedented among pairs of natural compounds; see, *e.g.*, the pyrethrins and cinerins. Other analogous, if less closely related instances of occurrence—sometimes co-occurrence — of pairs of compounds R-C_2 and R-C_1, where C_2 is ethyl rather than vinyl, can be found among the pyrromycinones and rhodomycinones.

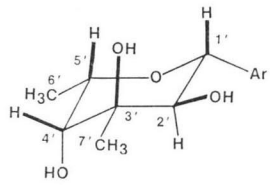
and chromatographically, with the antitumor antibiotics toromycin or gilvocarcin V, and gilvocarcin M, for which the structures and relative configurations shown below have been established.[5~7]* Evidently, structures **3** and **4** contain all of the features revealed for **1** and **2** by the ^{1}H and ^{13}C NMR signals ascribable to the aromatic moieties of these compounds; this fact further confirms the identity of the chromophores in **1** and **3**, and **2** and **4**, respectively.

The problem of establishing the complete structure and relative configuration of chrysomycins A and B (**1** and **2**, respectively) thus reduced itself to the elucidation of the side-chain, identical in both compounds. It was solved through application of ^{1}H and ^{13}C NMR spectroscopy, which led to the structure and relative configuration shown below. These spectra are documented in Tables 1, 2, and 3, and Fig. 1.

The methyl glycoside of a sugar virenose, apparently identical with, or antipodal to, the *C*-glycosidic moiety of **1** and **2**, has been obtained[8] through methanolysis of an antitumor antibiotic virenomycin.[9] The structure of the aglycone moiety of virenomycin has not yet been published, but UV data







(5)

(**1**) R = $CH{=}CH_2$ (**3**) R = $CH{=}CH_2$

(**2**) R = CH_3 (**4**) R = CH_3

The numbering system for the chromophores of **1**~**4** is the one given for the gilvocarcins, (**3**) and (**4**), in reference 5.

Table 1. ^{1}H NMR spectra of the chromophoric moieties of chrysomycin A (**1**) and gilvocarcin V (**3**) in DMSO-d_6; data on (**3**) from reference 5.

Proton	Chrysomycin A (**1**)	Gilvocarcin V (**3**)
2	7.24 (1H, d, 8.5 Hz)	6.92 (1H, d, 8.3 Hz)
3	8.16 (1H, d, 8.5 Hz)	8.05 (1H, d, 8.3 Hz)
7	8.12 (1H, d, 1.5 Hz)	7.93 (1H, d, 1.5 Hz)
9	7.69 (1H, d, 1.5 Hz)	7.69 (1H, b, s)
11	8.47 (1H, s)	8.39 (1H, s)
1 (O*H*)	9.50 (1H, s)	9.66 (1H, s)
10 (OCH_3)	4.28 (3H, s)	4.14 (3H, s)
12 (OCH_3)	4.25 (3H, s)	4.08 (3H, s)
Vinyl	5.71 (1H, d, 11.0 Hz)	
	6.28 (1H, d, 17.6 Hz)	6.11 (1H, d, 18.6 Hz)
	7.07 (1H, dd, 11.0 and 17.6 Hz)	6.93 (1H, dd, 9.2 and 18.6 Hz)

* The published spectrum[5] of toromycin suggests that this compound contains a small amount of the analog with a methyl instead of a vinyl group.

Fig. 1. ^1H NMR spectrum of chrysomycin A triacetate; 360 MHz, in $CDCl_3$ with TMS as internal standard.

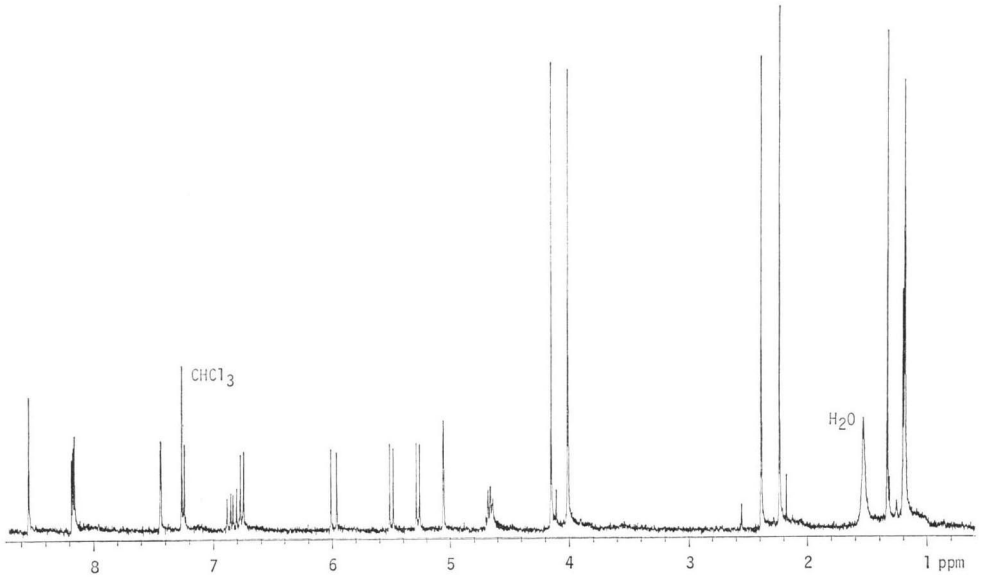
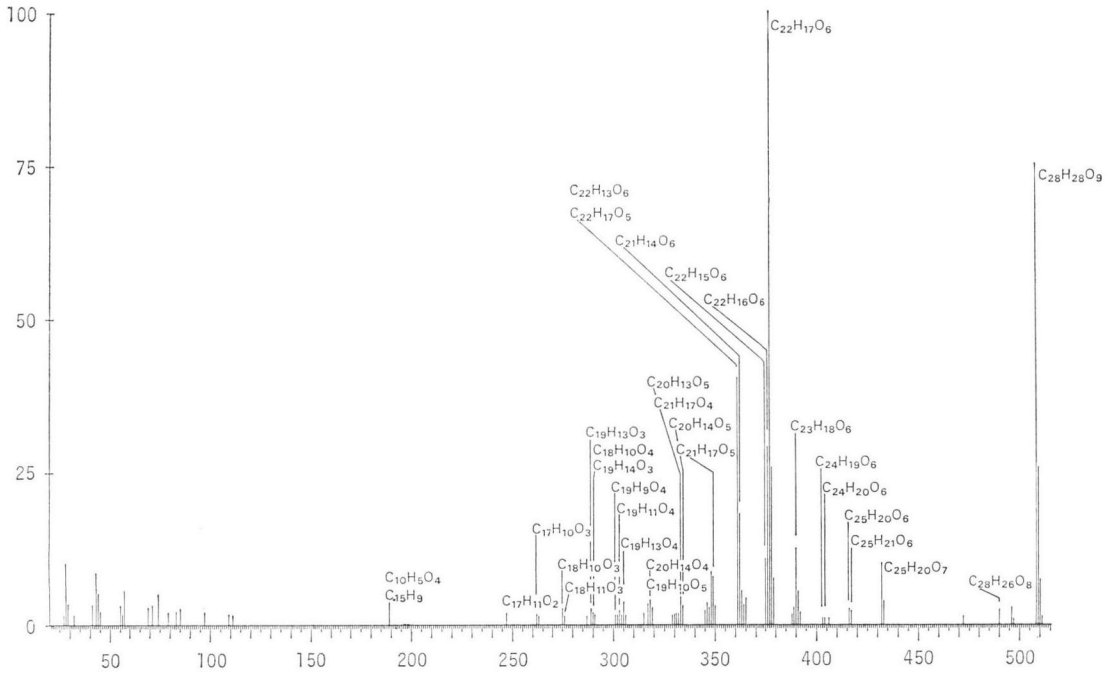


Fig. 2. Electron-impact mass spectrum of chrysomycin A (**1**).



suggest that it may be related to **5** and **6**.

The structure and stereochemistry of methyl β-D-virenoside have been confirmed recently by total synthesis.[10] Comparison of the ^1H NMR spectrum of the triacetate of **1** with the published spectrum of the diacetate of synthetic methyl β-D-virenoside shows close correspondence of the coupling constants,

Table 2. ^{13}C NMR spectra of the chromophoric moieties of chrysomycin A (**1**) and gilvocarcin V (**3**) in DMSO-d_6; data on (**3**) from reference 5.

Carbon	Chrysomycin A	Gilvocarcin V	Carbon	Chrysomycin A	Gilvocarcin V
1	153.1	152.5	9	113.8	113.8
2	112.0	111.7	10	157.2	156.8
3	128.1	128.7	10a	122.5	122.5
4	125.8	125.7	10b	112.0	112.5
4a	128.0	123.3	11	101.5	100.9
4b	142.3	141.9	12	151.7	151.3
6	157.7	159.3	12a	114.6	114.5
6a	121.9	121.6	10 (OCH_3)	56.7	56.2
7	119.0	118.7	12 (OCH_3)	56.2	55.7
8	138.6	138.1	Vinyl	116.3, 135.1	116.5, 135.0

Table 3. ^1H and ^{13}C NMR data for the carbohydrate moiety of triacetylchrysomycin A; Solvent $CDCl_3$.

^1H	Position	^{13}C*
6.76, 5.28 (J=9.5)	1′	74.7 (d)
	2′	73.1 (d)
—	3′	73.0 (s)
5.07 (J=1.2), 4.76 (J=7.0)	4′	75.8 (d)
	5′	70.6 (d)
1.20	6′	23.8 (q)
1.33	7′	17.0 (q)

* Multiplicities shown by off-resonance decoupling.

Table 4. Comparison of the ^1H NMR spectrum of triacetylchrysomycin A with that of synthetic methyl β-D-virenoside; solvent $CDCl_3$.

Triacetyl-chrysomycin A	Proton	Methyl β-D-virenoside (data from reference 10)
6.76, 5.28 (J=9.5 Hz)	1′	4.58, 4.81 (J= 8 Hz)
	2′	
5.07, 4.76 (J=1.2 Hz)	4′	4.80, 4.23 (J=1.2 Hz)
	5′	
1.20 (J=7.0 Hz)	6′	1.14 (J=6.5 Hz)
1.33	7′	1.12

while the chemical shifts of **1** triacetate reflect the presence of the aromatic system. See Table 4.

Molecular models of **1** triacetate indicate that the most probable conformations of the pyranose system place the sugar protons above the aromatic rings. As a consequence, the 2′-acetoxy group produces a signal at 1.19 ppm, 1.0 ppm above the one of the 4′-acetoxy group.

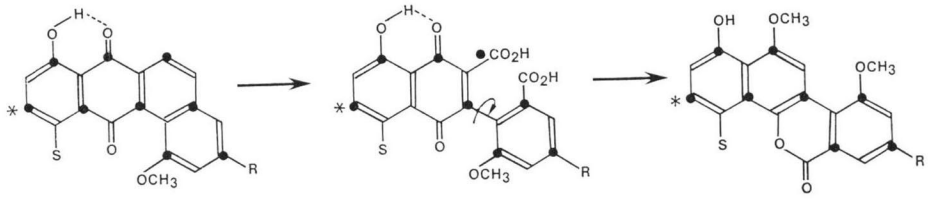
The *in vivo* antitumor activity of chrysomycin was determined in mice against P 388 lymphocytic leukemia under contracted accessory services in accordance with the National Cancer Institute guidelines for natural products.[11] The sample tested contained 86 percent chrysomycin A and 14 percent chrysomycin B. Supply limitations confined the testing to a single dose, administered intraperitoneally 24 hours after inoculation with the leukemia cells. The drug was suspended in hydroxypropyl cellulose. At 400 mg/kg chrysomycin produced an increase of the life span (ILS) of the treated mice of 54%, while exhibiting no lethal toxicity. These data are very similar to those (ILS 57%, LD_{50} > 1,000 mg/kg) reported[12] for gilvocarcin V.

The action of STRELITZ and FLON's chrysomycin against a number of bacteria, molds, and bacteriophages is discussed in reference 1.

The biosynthesis of the chromophoric moieties of antibiotics **1**~**4** presents an interesting problem. As indicated in diagram **6**, these structures are undoubtedly of polyketide origin, but they can hardly be formed completely by simple folding of one single acetate-malonate-derived carbon chain. A formation from two such chains would have a precedent *e.g.* in the cases of citromycetin[13] and mollisin[14,15,16];

alternatively, these structures could be interpreted as arising from one chain (as is the case with the vast majority of polyketides of known biosynthetic origin), if the group R were a secondary addition—an assumption which is plausible enough for $R=CH_3$, but hardly for $R = CH=CH_2$.

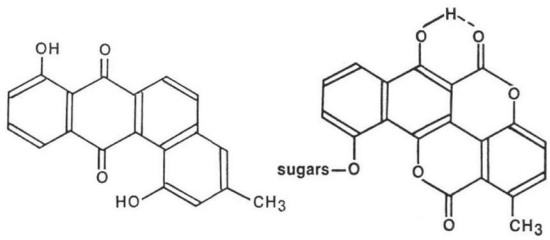
In the absence of experimental evidence, we prefer to assume formation of these structures by oxidative cleavage of a 1,2-benzanthraquinone precursor such as **7**, with subsequent unexceptional changes: decarboxylation to **8**, reduction, lactonization, and *O*-methylation, as shown below:



(7) **(8)** **(6)**
(=**1**~**4**)

R = $CH=CH_2$ or CH_3
S = sugar side-chain

Several microbial metabolites with the ring system of **7** are known; one of them, tetrangulol, **9**[17], actually differs from **7**, $R=CH_3$, only through the absence of the *O*-methyl and the *C*-glycosidic side-chain. Other compounds of this type[18] are tetrangomycin,[17] ochromycinol, rabelomycin, and aquayamycin (this last one a *C*-glycoside!). It is striking that all these structures, including **1**~**4**, lack the oxygenated function in the marked (*) position of the ring system, which would be expected to be present on the basis of the polyketide origin.



(9) **(10)**

A similar biosynthesis has been discussed[19], as one of several possibilities, for the antibiotic chartreusin, **10**, whose structure[20] bears an unmistakable resemblance to those of compounds **1**~**4**.

Experimental

Materials and Methods

All the chrysomycin used had been isolated by STRELITZ and FLON[1] during the initial work on this antibiotic. The material consisted of a number of small samples, all but one of which were found to be mixtures of much chrysomycin A (**1**) with varying smaller amounts of chrysomycin B (**2**). The one exceptional sample, shown by HPLC to consist of ~98% pure **1**, served for the analytical and spectroscopic characterization of this compound.

NMR spectra (electron impact) were recorded on the JEOL FX-60 and NTC-360 instruments of the National Heart, Lung, and Blood Institute, with TMS as internal standard. IR spectra were taken in KBr pellets on a Beckman Model 4230 instrument. Melting points were taken on a Reichert hot-stage microscope; they are not corrected for stem exposure. High-resolution mass spectra were taken on an MS-2902 instrument.

Chrysomycin B (2)

Only minute amounts of the pure compound have been obtained so far. The UV and visible spectrum of **2** agrees with the one of gilvocarcin M shown in Fig. 1 of reference 6. The pattern of the mass-spectrometric fragmentation of **2** closely resembles that of **1**, with the corresponding peaks occurring at

mass values lower by 12 units, as expected. For the parent peak, *m/z* 496.1741 was found; Calcd. for $C_{27}H_{28}O_9$. 496.1733. The ^1H and ^{13}C NMR spectra closely resemble those of **1**, except for the replacement of the signals from the protons and carbon atoms of the vinyl group by those of methyl on the aromatic ring: ^1H NMR, δ 2.51; ^{13}C NMR, δ 20.94.

Chrysomycin (STRELITZ and FLON), Acetylation, and Separation of the Resulting Product into Triacetylchrysomycins A and B

The old sample (~90% A and 10% B, by HPLC), 4 mg, was dissolved in 1 ml pyridine, and acetic anhydride (1 ml) was added dropwise with stirring. The mixture was stirred at room temperature for 24 hours. It was next concentrated under reduced pressure, and the residue was taken up in $CHCl_3$ (1 ml). The triacetates of **1** and **2** were separated on a C-18 Magnum 9 column (Whatman Inc.) with methanol - H_2O - tetrahydrofuran (50: 35: 15) as mobile phase. The retention times were 17.5 minutes for the triacetate of chrysomycin A, and 14.5 for that of B. Mass spectrometry confirmed the presence of three acetyl groups.

Triacetylchrysomycin A

Mp 311~312°C. MS: *m/z* 634.1965 (M^+) (Calcd. for $C_{34}H_{34}O_{12}$: 634.2048). UV $\lambda_{max}^{CDCl_3}$ (log ε) 217 (3.97), 251 (4.47), 265 (4.36), 307 (4.53), 325 (4.16), 337 (4.06), and 392 (4.10) nm. IR ν_{KBr} 3500, 1725 cm^{-1}. The ^1H NMR spectrum shows peaks very similar to those of the non-acetylated compound, with the exception of those caused by the protons on the carbohydrate moiety (see Table 3), and of the three *O*-acetyl groups (δ 1.20, 2.28, 2.42, s, 3H). The low δ-value of one of these (also observed in tetraacetylgilvocarcin V[6] and tetraacetyltoromycin[5]) is undoubtedly caused by the influence of the aromatic chromophore; this circumstance localizes[5] this particular acetoxy group on C-2′.

Triacetylchrysomycin B

Mp 205~207°C. MS: *m/z* 622.2033 (M^+) (Calcd. for $C_{33}H_{34}O_{12}$: 622.1749). UV $\lambda_{max}^{CDCl_3}$ (log ε) 214 (3.83), 248 (4.64), 278 (4.52), 307 (4.64), 326 (4.10), 339 (4.13), and 382 (4.08) nm. IR ν_{KBr} 3550, 1725, 1230 cm^{-1}. As expected, the ^1H and ^{13}C spectra of the compound are very similar to those of triacetylchrysomycin A, except again for the replacement of the signals from the vinyl group of triacetylchrysomycin A by those of methyl on an aromatic ring. ^1H NMR δ 2.51; ^{13}C NMR δ 21.6.

Addendum

After completion of this manuscript, papers describing two more antibiotics closely related to **1**~**4** have come to our knowledge. Like **1**~**4**, these new antibiotics are *C*-glycosides.

From fermentation broths of *Streptomyces ravidus,* FINDLAY *et al.*[21] have isolated the antitumor antibiotic ravidomycin, in which the chromophore of **1** and **3** carries an amino sugar, *viz.* 4-*O*-acetyl-3, 6-dideoxy-3-dimethylamino-pseudo-altropyranose. Another species, *S. anandii*, was found by BALITZ *et al.*[22] to produce **3** and **4**, together with the new gilvocarcin E, in which the vinyl group of **3** is replaced by ethyl. It was observed[22] that all three antibiotics elaborated by *S. anandii* have antimicrobial action, but that antitumor activity is restricted to gilvocarcin V (**3**).

Acknowledgments

The authors are much indebted to Dr. MARJORIE ANCHEL, New York Botanical Garden, for making available the samples of chrysomycin, and to Dr. PETER P. ROLLER and Mr. J. R. MILLER, National Cancer Institute, for the mass spectra.

References

1) STRELITZ, F.; H. FLON & I. N. ASHESHOV: Chrysomycin: a new antibiotic substance for bacterial viruses. J. Bacteriol. 69: 280~283, 1955
2) For an interesting review of this work, see ASHESHOV, I. N.; E. HALL & H. FLON: A survey of actinomycetes for antiphage activity. Antibiotics & Chemother. 4: 380~394, 1954
3) WEI, T. T.; J. A. CHAN, P. P. ROLLER, U. WEISS, R. H. STROSHANE, R. J. WHITE & K. M. BYRNE: Detection of gilvocarcin antitumor complex by a biochemical induction assay (BIA). J. Antibiotics 35: 529~532, 1982

4) ELESPURU, R. H. & M. B. YARMOLINSKY: A colorimetric assay of lysogenic induction designed for screening potential carcinogenic and carcinostatic agents. Environmental Mutagenesis 1: 65~78, 1979
5) HORII, S.; H. FUKASE, E. MIZUTA, K. HATANO & K. MIZUNO: Chemistry of toromycin. Chem. Pharm. Bull. 28: 3601~3611, 1980
6) TAKAHASHI, K.; M. YOSHIDA, F. TOMITA & K. SHIRAHATA: Gilvocarcins, new antitumor antibiotics. 2. Structural elucidation. J. Antibiotics 34: 271~275, 1981
7) HIRAYAMA, N.; K. TAKAHASHI, K. SHIRAHATA, Y. OHASHI & Y. SASADA: Crystal and molecular structure of the antibiotic gilvocarcin M. Bull. Soc. Chem. Japan 54: 1338~1342, 1981
8) KULYAEVA, V. V.; M. K. KUDINOVA, N. P. POTAPOVA, L. M. RUBASHEVA, M. G. BRAZHNIKOVA, B. V. ROZYNOV & A. R BEKKER: Structure of the carbohydrate moiety of the antibiotic virenomycin. Bioorg. Khim. 4: 1087~1092, 1978; Chem. Abstr. 90: 104,282t, 1979
9) BRAZHNIKOVA, M. G.; M. K. KUDINOVA, V. V. KULYAEVA, N. P. POTAPOVA & V. I. PONOMARENKO: Physicochemical characteristics of virenomycin, a new antitumorigenic antibiotic. Antibiotiki 22: 967~970, 1977
10) YOSHIMURA, T.; N. HONG & K. I. SATO: Synthesis of methyl β-D-virenoside. Chem. Lett. 1980: 1131~1132, 1980
11) GERAN, R. I.; N. H. GREENBERG, M. M. MACDONALD, A. M. SCHUMACKER & B. J. ABBOTT: Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemother. Rep. Part 3, 3: 7~61, 1972
12) NAKANO, H.; Y. MATSUDA, K. ITO, S. OHKUBO, M. MORIMOTO & F. TOMITA: Gilvocarcins, new antitumor antibiotics. 3. Antitumor activity. J. Antibiotics 34: 701~707, 1981
13) GATENBECK, S. & K. MOSBACH: The mechanism of the biosynthesis of citromycetin. Biochem. Biophys. Res. Commun. 11: 166~169, 1963
14) BENTLEY, R. & S. GATENBECK: Naphthoquinone biosynthesis in molds. The mechanism of the formation of mollisin. Biochemistry 4: 1150~1156, 1965
15) TANABE, M. & H. SETO: Biosynthetic studies with carbon-13: mollisin. Biochemistry 9: 4851~4853, 1970
16) SETO, H.; L. W. CARY & M. TANABE: Utilization of ^{13}C-^{13}C coupling in structural and biosynthetic studies; the Fourier transform ^{13}C nuclear magnetic resonance spectrum of mollisin. Chem. Commun. 1973: 867~868, 1973
17) KUNTSMANN, M. P. & L. A. MITSCHER: The structural characterization of tetrangomycin and tetrangulol. J. Org. Chem. 31: 2920~2925, 1966
18) DEVON, T. K. & A. I. SCOTT: Handbook of Naturally Occurring Compounds. Vol. I. Acetogenins, Shikimates, and Carbohydrates. p. 371. Academic Press, New York, 1975
19) BROWN, J. R.; M. S. SPRING & J. R. STOKER: Biosynthesis of the aglycone of chartreusin in *Streptomyces* sp. X-465. Phytochemistry 10: 2059~2064, 1971
20) SIMONITSCH, E.; W. EISENHUTH, D. A. STAMM & H. SCHMID: Ueber die Struktur des Chartreusins. Helv. Chim. Acta 43: 58~63, 1960; 47: 1459~1475, 1964
21) FINDLAY, J. A.; J.-S. LIU, L. RADICS & S. RAKHIT: The structure of ravidomycin. Can. J. Chem. 59: 3018~3020, 1981
22) BALITZ, D. M.; F. A. O'HERRON, J. BUSH, D. M. VYAS, D. E. NETTLETON, R. E. GRULICH, W. T. BRADNER, T. M. DOYLE, E. ARNOLD & J. CLARDY: Antitumor agents from *Streptomyces anandii*: gilvocarcins V, M and E. J. Antibiotics 34: 1544~1555, 1981