Synthesis of Furanomycin Analogs

by

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Chemistry

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Approved by:

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Abstract

The synthesis of the dihydro- and 7-Halodihydroanalogs of the antibiotic, antimetabolite Furanomycin was investigated. Stereochemistry of the resulting compounds was compared to Furanomycin. Initial biological testing of the analogs was conducted.

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Background

The incidence of cancer has increased dramatically in recent decades due both to natural and man-made factors. Traditional methods of carcinoma treatment, radiation therapy and surgery, have been found to have limited effectiveness. Recently new applications of these methods coupled with chemotherapy have yielded significantly improved results. The use of multiple drug combinations along with radiation therapy and surgery has recently been tried with good results.[1] This has initiated investigations in the development of newer and more active chemotherapeutic agents.

One area of this investigation has involved the extensive examination of natural products isolated from plant and animal sources. Many of the agents currently used in cancer chemotherapy have been derived from natural sources.[1] However, investigation and application of many natural products is limited because there are insufficient quantities of pure materials available from natural sources. A second area of investigation has involved evaluating the biological activity of analogs of natural products. This is

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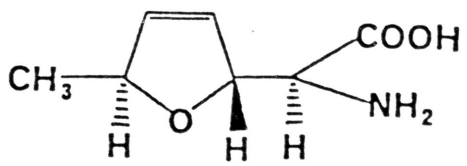
an important area because many analogs show an increased activity and/or decreased toxicity.

This project is directed toward the synthesis and initial biological testing of analogs of the antibiotic Furanomycin (1). Furanomycin has been isolated from the culture filtrate of Streptomyces L-803.[2] It inhibits the growth of the Coliphage T-2[2] and has been shown to have a higher inhibitory effect on microorganisms in a synthetic media than in nutrient agar.[2] Furanomycin has also been found to be an L-Isoleucine antagonist.[2]

The structure of Furanomycin puts two requirements on the synthesis of its analogs. Furanomycin has a furan ring with the methyl and α-amino acid functionalities in a trans configuration.[3] The α carbon and carbon 2 have a threo relationship with a complete molecular configuration of 5S, 2R, αS.[3] Analogs of Furanomycin will be synthesized with a trans stereochemistry across the furan ring but will be a mixture of the threo and erythro isomers.

Two compounds of interest are the dihydro-(2) and 7-Iododihydro-(3) analogs of Furanomycin. Upon synthesis of these compounds a comparison of their biological activity with Furanomycin can be made. These compounds are also of interest because of structural similarity with compounds related to the antineoplastic antibiotic 593A (4).

593A is the first known naturally occurring nitrogen mustard. It was isolated in 1970 from the fermentation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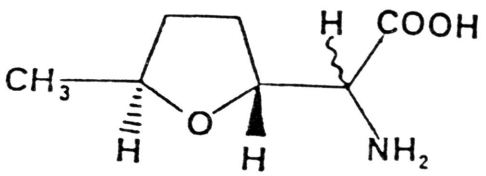
NH2
O
H
H
H

1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O
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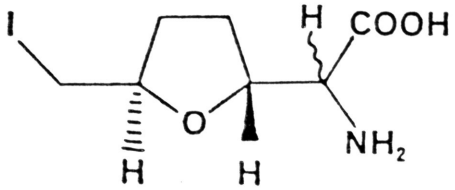
H
NH2

2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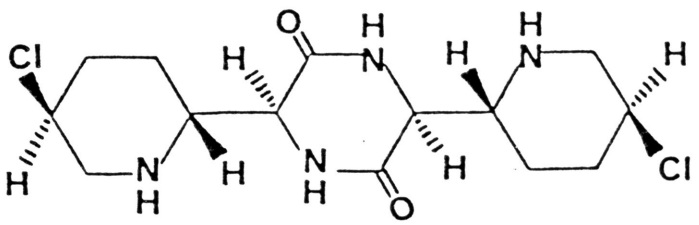
H
COOH
O
H
H
NH2

3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H
N
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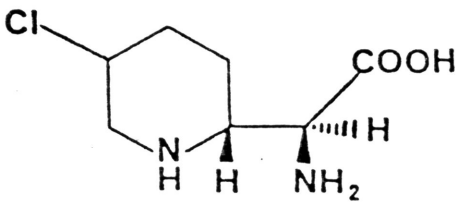
N
H
Cl
H
H
N
H
N
H
H
Cl
O

4

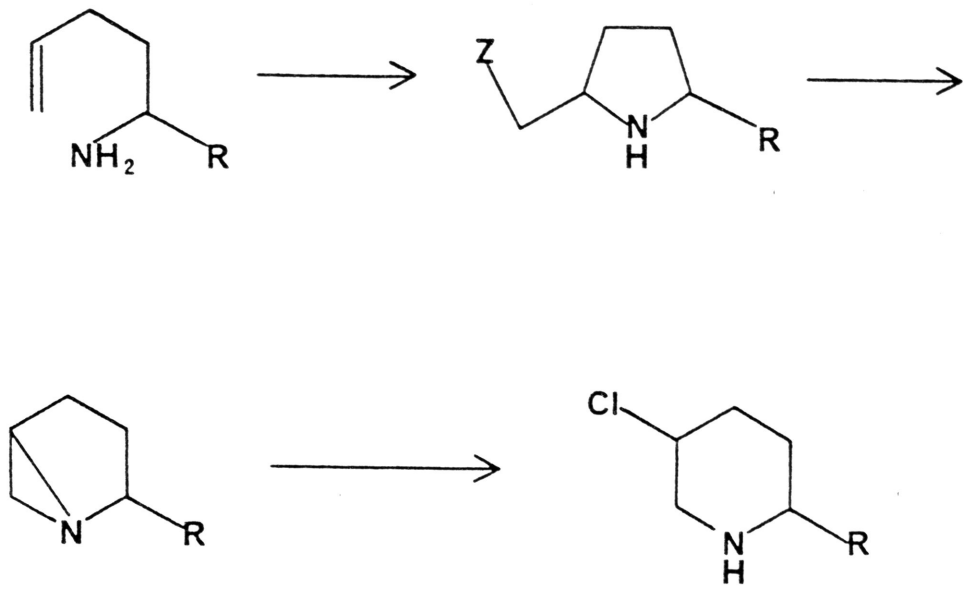

Cl
COOH
H
N
H
H
NH2

5

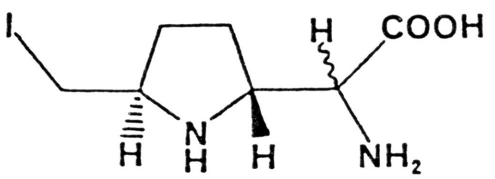
broth of Streptomyces griseolutus at Merck and Co.[4] and the structure 4 was determined in 1976 by x-ray analysis.[5] 593A has been shown to inhibit the growth of a variety of neoplastic cell lines,[6] and has been brought to chemical trial (NSC-135758) with encouraging results.[6]

593A can be considered a dimer of the amino acid streptolutine[7] (5). The synthesis of 5 has been proposed as shown (Scheme 1).[8] The halomethyl pyrrolidine (6) and the methyl pyrrolidine (7) can be seen to be nitrogen analogs of the Furanomycin analogs 2 and 3. The biological activity of 6 and 7 will be measured and compared to that of furanomycin and its analogs.

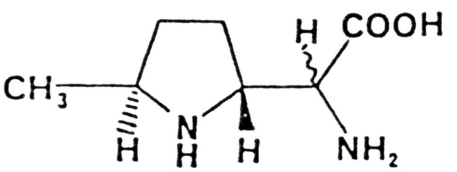
It is anticipated that 3 may show some antineoplastic activity since 3 has a halogen β to a hetero atom which is analogous to the nitrogen mustard functionality. Nitrogen mustards have long been recognized as a class of compounds with widespread utility in chemotherapy treatment.[9] This relationship may allow 3 to possess antineoplastic activity while reducing the toxicity associated with nitrogen mustards. Antineoplastic activity has been related to the biological activity of a compound in some bacterial strains,[10] so that initial biological testing can give indications of chemotherapeutic activity.



Scheme 1



6



7

Synthesis

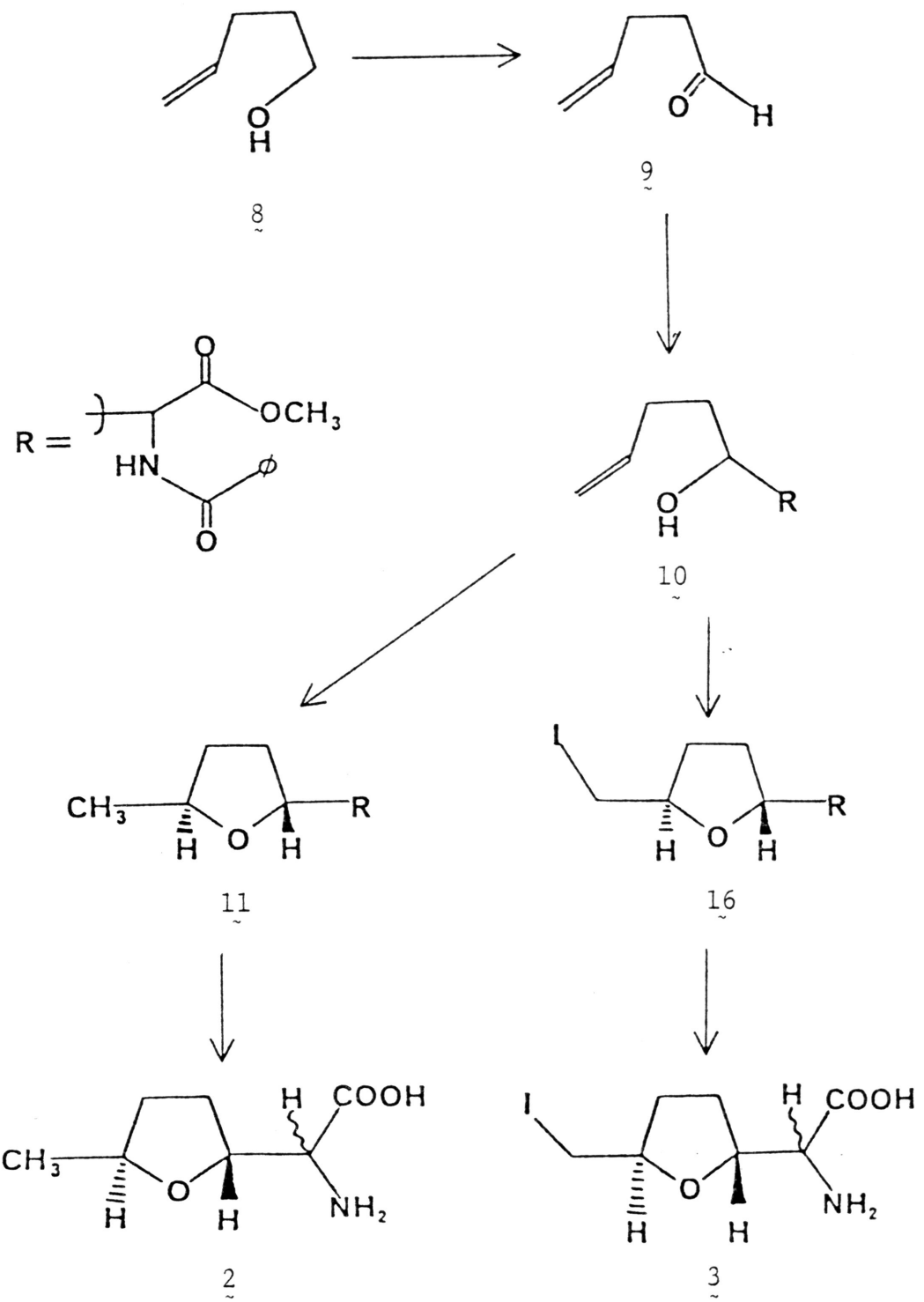
As stated previously, Furanomycin contains the trans stereochemistry across the furan ring and a threo relationship between carbon 2 and α. The proposed synthesis, which insures the trans relationship in the furan ring, is shown below. (Scheme 2)

4-Penten-1-ol (8), which is commercially available at 98% purity, was oxidized to the aldehyde (9) in the first step of this synthesis. Mandapur's procedure[11] was followed yielding pure 9 in 32% yield.

Hydroxyalkylation of methyl hippurate (13) with aldehydes related to 9 has been reported.[12] Mechanistically, this reaction entails formation of the lithiodianion of 10 followed by nucleophilic attack of the α carbanion at the aldehyde carbonyl. (Scheme 3) This reaction with 9 gave 10 in 20.5% yield.

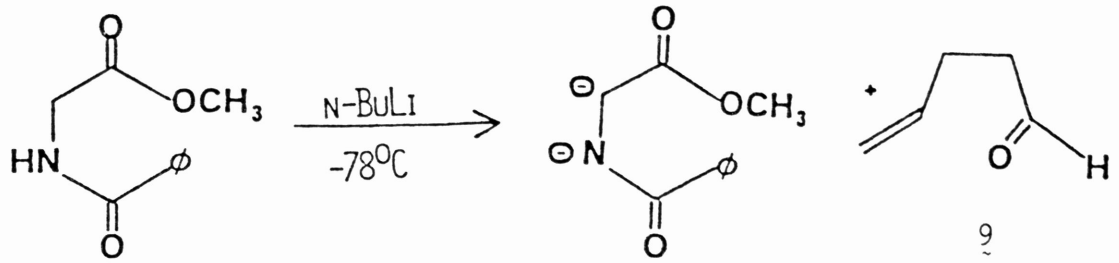
Scheme 4 presents an alternative method to obtain 10. The acylation of 14 with methyl hippurate, following Harding's procedure,[12] gave the β-keto ester 15 in 35% yield. Reduction of this following Shapiro's procedure[13] yielded the desired β-hydroxy ester (10) in 93% yield.

The hydroxyalkylation of 9 and the reduction of 15 yields 10 as a mixture of isomers. Compound 10 is generated as a mixture of diastereomers with relative stereochemistry of either erythro or threo about carbons 2 and 3. Each

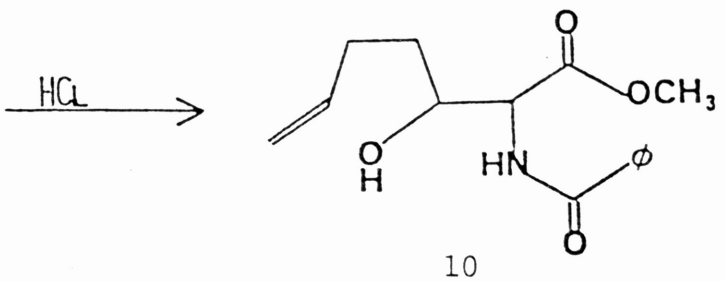


8 9 10 11 16 2 3

Scheme 2

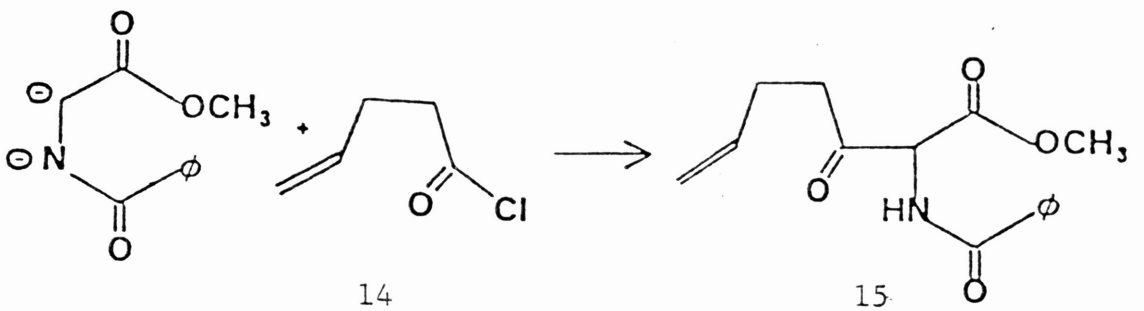


13 9

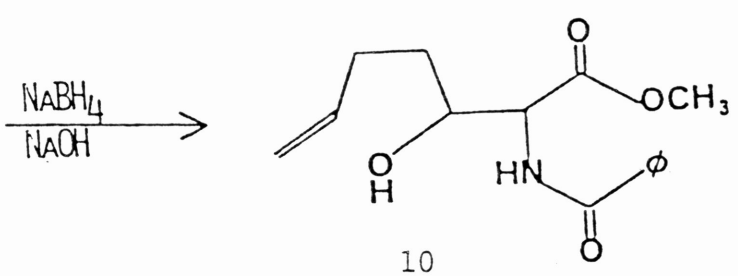


10

Scheme 3



14 15



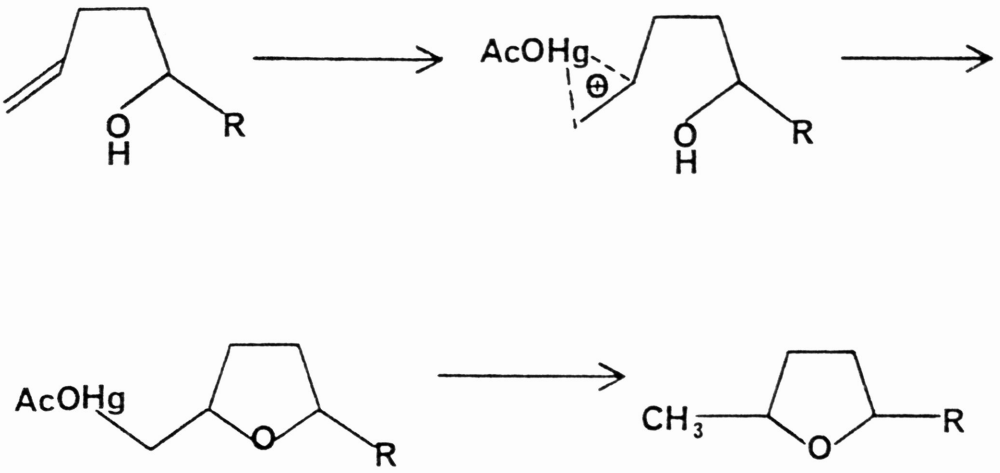
10

Scheme 4

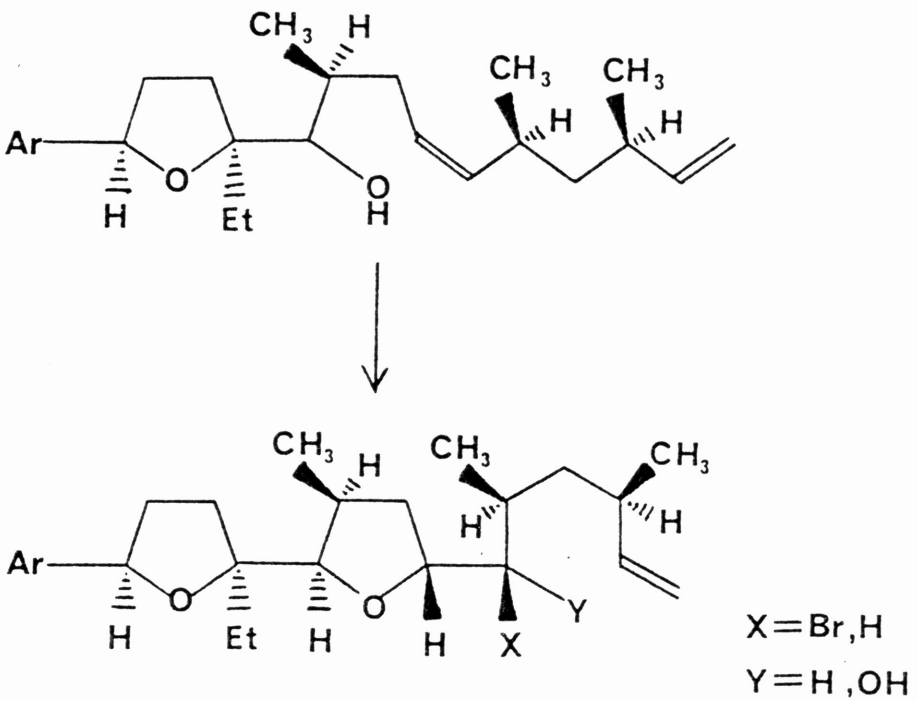
diastereomer will consist of a racemic mixture of enantiomers. Separation of the diastereomers would be possible either at this point or after cyclization, but this was not done in this study.

Compound 11 was obtained by the intramolecular alkoxymercuration of 10 followed by reduction using Brown's procedure.[14] The cyclization reaction involves formation of the mercurinium ion across the double bond followed by intramolecular cyclization to a 5 membered ring. (Scheme 5) Sodium borohydride reduction of the mercuric acetate functionality leaves a methyl group on the tetrahydrofuran ring. This reaction sequence gave 11 in 88% yield.

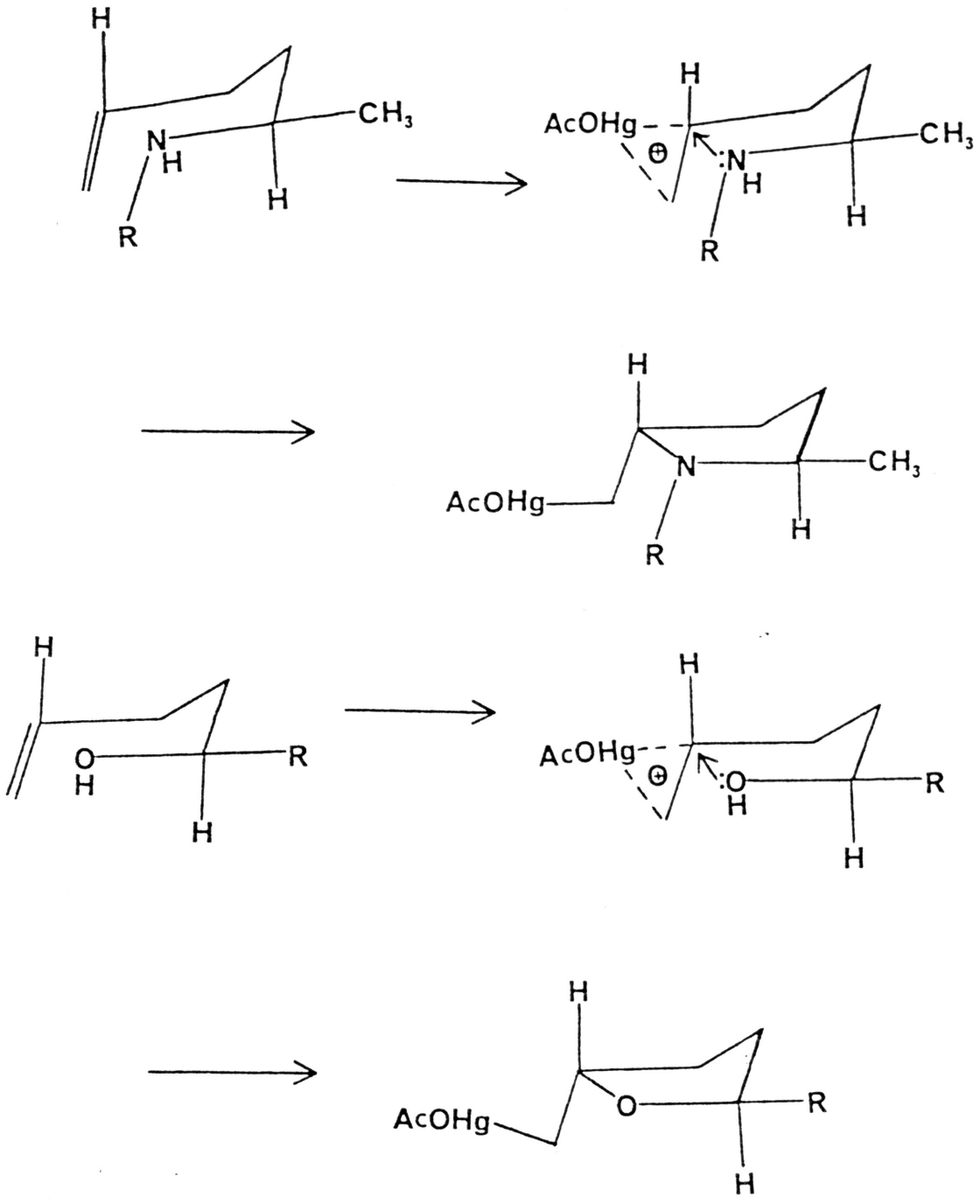
Proton NMR of the product showed two methoxy singlets of unequal intensity and a methyl doublet, indicating that the cyclization occurred stereospecifically across the furan ring, giving two products differing in the relative configuration at the α carbon. If the erythro and threo isomers of 10 were separated, each should give a single product upon cyclization. The stereochemistry across the furan ring can be predicted with certainty based upon analogy with reactions conducted with prostagladins as substrates[15] (Scheme 6) and with a simple amidomercuration cyclization.[16] Scheme 7 shows a mechanistic comparison of the two systems. Both unsaturated starting materials are shown in the chair conformation with the attached substituents in the more stable equatorial position. Formation



Scheme 5



Scheme 6


H
CH3
N
H
H
R
AcOHg
⊕
:N
H
H
R
CH3
H
AcOHg
N
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R
H
O
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R
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AcOHg
⊕
:O
H
R
H
H
AcOHg
O
R
H

Scheme 7

of the mercurinium ion followed by intramolecular cyclization yields the trans stereochemistry in both cases. It appears then that the cyclization has been achieved with the desired trans stereochemistry.

Cyclization of 10 will have no effect on the stereochemistry of C2 and Cα. The ratios of relative and absolute vs stereochemistry of these two carbons will be unaffected. If the diastereomers had been separated prior to this cyclization then the threo isomer would yield the threo cyclized product. If a mixture of diastereomers was used in cyclization then separation of them would be possible at this point.

Compound 16 was obtained by the iodocyclization of 10 in 50% yield following Johnson's procedure.[17] Proton NMR indicates that the reaction yielded only one isomer with respect to the furan ring. This would appear to be the trans isomer by the above arguments. Again, the stereochemistry of carbons 2 and α would be unchanged by this cyclization. A complex multiplet at 3.0-3.33 ppm, corresponding to the iodo-methylene, rather than a simple doublet indicates these prochiral protons have slightly different chemical shifts.

Compound 2 was obtained by removal of the methoxy and benzoylamide protecting groups from 11 by acid hydrolysis. The hydrolysis of 11 yielded 36% of 2. The hydrolysis product retained the trans stereochemistry across the furan

ring and a mixture of erythro and threo diastereomers with respect to carbons 2 and α. Compound 3 resulting from the hydrolysis of 16 has not been isolated at this time.

Biological Testing

The final phase of this project entails the initial biological testing of 2 and 3 to contrast their biological activity with Furanomycin and 7. This initial testing will be conducted using E. Coli, a Gram negative bacteria, and Bacillus subtilitus, a Gram positive bacteria. Both bacterial strains have been used as screens for anti-cancer drugs,[10] and biological activity against these bacteria may indicate anti-neoplastic activity.

These compounds will also be tested to see if they retain the anti-metabolite behavior of the Furanomycin. Tests comparing the biological activity of 2 and 3 in media containing L-isoleucine and deprived of L-isoleucine will be made. If L-isoleucine anti-metabolite behavior is present in 2 and 3, biological activity will be greater in the nutrient deprived medium.

Mutagenic behavior of 2 and 3 will also be investigated. Bacterial strains possessing mutations which prevent growth in certain media will be used to test for mutagenic activity. Significant growth of these bacteria in the presence of 2 or 3 will indicate that the compounds have induced a mutation in the bacterial gene pool.

Currently results from the biological testing phase of the project are unavailable.

Summary

This project was directed toward the synthesis and initial biological testing of the dihydro- and 7-Iodo-dihydro-analogs of Furanomycin. Both compounds have been synthesized with fair overall yields. Overall yields should be increased in future preparations since several steps of the synthesis are known to go in greater yields than reported here. Purification procedures for the products of each step were previously untried on these compounds. Now that techniques for purification are known, loss of product in unsuccessful attempts at purification should be significantly decreased. Since both reaction and purification protocols have been established, overall yields should be increased.

An important aspect of the synthesis of 2 and 3 is the stereochemistry of the resulting compounds. The procedures used here have resulted in compounds having a trans stereochemistry across the furan ring which corresponds to the stereochemistry of Furanomycin. The compounds synthesized here are a mixture of diastereomers about carbons 2 and α. The synthesis is stereoselective but it is not known whether the threo or erythro diastereomer predominates. These diastereomers can be separated either before or after cyclization to obtain the single isomers. To obtain the compound with the same absolute

stereochemistry as Furanomycin resolution of the threo racemic mixture must be achieved.

Biological testing is currently underway; however, results are currently available.

Experimental

Thin-layer chromotography (TLC) was performed by using plastic sheets which were precoated with a 0.25-mm layer of silica gel 60-F-254 (EM Reagents). Preparative TLC was carried out by using 20cm x 20cm glass plates coated with a 1.5-mm layer of silica gel (EM Reagents silica gel PF-254). Column chromatography was performed using Baker Chemical silica gel (60-230 mesh). Dry tetrahydrofran (THF) was distilled from the sodium benzophenone dianion prior to use. Solvent grade ether was used for extractions. Dry methylene chloride was obtained by gravity filtration of reagent grade CH_2Cl_2 through activated alumina. Brine refers to a saturated aqueous solution of sodium chloride.

Nuclear Magnetic Resonance (NMR) spectra were determined on a Varian Associates Model T-60, EM-360, EM-390, or XL200 spectrometer. Tetramethylsilane (Me_4Si) was used as the internal reference for all spectra except where stated. Otherwise, all chemical shifts are reported in parts per million (ppm) downfield from Me_4Si (δMe_4Si 0.0).

A dry Argon atmosphere was obtained using an Ale-Burlitch inert atmosphere system, which removed traces of oxygen and carbon dioxide by use of BASF catalyst.

Preparative high pressure liquid chromatography (HPLC) was performed on a Waters Prep 500 operating writer two silica cartridges at a flow rate of 200 ml/min.

4-Penten-1-al (9). A solution of 200 ml of dry CH_2Cl_2 and 16.21 g (0.075 mol) of pyridinium chlorochromate was stirred for 10 min in a flame-dried, 3-neck, Morton flask fitted with overhead stirring. Then 4.33 g (0.050 mol) of 4-penten-1-ol was added to the stirred solution. This was allowed to react for 2 h and then the mixture was diluted with 200 mL of anhydrous ether. The resulting solution was vacuum filtered through 40 g of Florisil and the solvent was removed by distillation through a Vigreux column. The product was dissolved in ether and vacuum filtered through Florisil. The filtrate was distilled (b.p. 94-108° C) to yield 1.33 g (32%) of the pure aldehyde. ^{1}H-NMR-T-60-$CDCl_3$-δ-2.3-2.5(M,4H,2X-CH_2-), 4.8-5.2(M,2H,=CH_2), 5.5-6.2(M,1H,=CH-), 9.75(S,1H,O=CH).

Methyl 2-benzoylamido-3-oxo-6-heptenoate (15). Acylation of the above β-keto ester was performed in the same manner as the preparation of 10 using 0.6 mL (~5.7 mmol) of 4-pentoyl chloride with the following change: the reaction was quenched with 2N HCl 5 min after the addition of 4-pentoyl chloride. The product was purified by chromatography on 50 g of silica gel eluted with 3:1 ether-hexane. This product was then further purified by HPLC eluted with 80:20 petroleum ether-ethyl acetate yielding 0.55 g (35%) of pure β-keto ester. ^{1}H-NMR-EM390-$CDCl_3$-δ-2.2-2.5(m,2H,-CH_2-C=), 2.8-3.0(m,2H,O=C-CH_2), 3.8(S,3H,OCH_3), 4.8-5.2-

(m,2H,=CH_2), 5.4(d,1H,CH), 5.5-6.0(m,1H,=CH), 7.2-7.5 and 7.7-7.9(m,6H,Aryl and NH).

Methyl 2-benzoylamido-3-hydroxy-6-heptenoate (10). Method A. Hydroxyalkylation of 9. A solution of 250 mL of dry THF, in a flame-dried, round-bottom flask equipped with magnetic stirring under dry Argon, was cooled to -78°C. All solutions were added using dried gas-tight syringes. To this solution was added 2.36 mL (15.52 mmol) of tetramethylethylene diamine followed by 9.75 mL (15.52 mmol) of 1.6 M n-butyllithium. Then 3.24 mL (15.52 mmol) of hexamethyl disilazane was added slowly and the solution was allowed to stir for 10 min. Methyl hippurate (1.51 g, 7.8 mmol) was added to the solution in 10 mL of dry THF, and stirring was continued for 10 min. Then 4-penten-1-al (0.674 g, 8.03 mmol) was added quickly to the solution and it was allowed to stir for 2 h. The solution was allowed to warm to 0°C and the reaction was quenched with 20 mL of 2N HCl. The solution was then concentrated, dissolved in ether, washed with H_2O and brine, dried over magnesium sulfate, filtered, and concentrated. The product was chromatographed by HPLC using ethyl acetate. The major component was further purified by chromatography on a column of 50 g of silica gel eluted with 3:1 petroleum ether - ethyl acetate, and monitored by TLC. The second fraction yielded 0.444 g (20.5%) of pure β-hydroxy ester.

^{1}H-NMR-EM390-$CDCl_3$-δ-1.4-1.8(m,2H,C<u>H</u>$_2$-CH), 1.9-2.4(m,2H,CH_2-C=), 3.7(2S,3H,OMe), 3.3-4.3(br-m,2H,<u>H</u>O-C<u>H</u>), 4.7-4.8(dd,1H-O=C-CH), 4.8-5.1(m,2H,=CH_2), 5.5-6.0(m,1H,=CH), 7.1-7.5 and 7.7-7.8(m,6H,Aryl and NH).

<u>Method B. Reduction of 15</u>. A solution of 0.3455 g (1.3 mmol) of 15 in 30 mL of methanol was cooled to 10-15°C in an ice bath. Then 0.025 g (0.79 mmol) of $NaBH_4$ in 1 mL of H_2O and 1 drop of 2.5 MNaOH was added to the solution. This was stirred for 30 min, and then poured into a mixture of 20 mL of ice water and 20 mL of saturated salt solution. This was extracted with ether (3 X 20 mL). The organic phases were washed with H_2O (3 X 15 mL), dried, filtered, and concentrated. This yielded 0.33g (93%) of pure β-hydroxy ester.

<u>Methyl 2-benzoylamido-2-(4-methyl-tetrahydrofuran-2-yl)-acetate (11)</u>. To a stirred 25 mL solution of THF in a Morton flask, was added 1.02 g (3.2 mmol) of mercuric acetate. This was stirred for 10 min, and then 0.44 g (1.6 mmol) of (10) was added. The solution was wrapped in foil and allowed to stir overnight. The reaction was quenched with 0.08 g (2.1 mmol) of sodium borohydride dissolved in a minimum of a 2.5N NaOH solution. This was stirred until mercury metal had formed at the bottom of the flask (2 h). The solution was concentrated and extracted 3 times with 15 mL of ether. The organic phases

were washed with brine and H_2O, dried over magnesium sulfate, filtered, and concentrated. This yielded 0.39 g (88%) of pure 11.

Methyl 2-benzoylamido-2-(4-iodomethyl-tetrahydrofuran-2-yl)-acetate (16). To a stirred solution of 50 mL of dry CH_2Cl_2 in an ice bath was added 0.237 g (0.93 mmol) of iodine and 0.076 g (0.72 mmol) of Na_2CO_3. This was stirred for 10 min. Then 0.128 g (0.46 mmol) of 10 was added in dry CH_2Cl_2 and the solution stirred for 2 h. The mixture was extracted with sodium bisulfite (3 X 2 mL), dried, filtered, and concentrated. The crude product was purified by preparative TLC yielding 0.092 g (50%) of pure 16.

Dihydro furanomycin (2). To a 5 mL pear-shaped flask equipped with magnetic stirring and a reflux condensor was added 0.100 g (0.36 mmol) of 11 and 0.964 g (026.1 mmol) of concentrated HCl. This solution was refluxed overnight in an oil bath. The solution was concentrated to remove excess HCl, dissolved in 8 mL ethanol, filtered, and taken to pH 7 with 1 drop of concentrated NH_4OH. The crystals were filtered off and the filtrate concentrated. This yielded 0.007 g (36%) of (2) from the filtrate.

References

1. G. R. Pettit, Vol. 1 Biosynthetic Products for Cancer Chemotherapy, Plenum Press, N.Y. and London, (1977) p. 29.

2. K. Katagiri, K. Tori, Y. Kimura, T. Yoshida, T. Nagasaki, H. Minato, J. Med. Chem., 10, 1149 (1967).

3. M. Shiro, H. Nakai, K. Tori, J. Nishikawa, Y. Yoshimura, K. Katagiri, J. C. S. Chem. Comm., 375 (1980).

4. C. O. Gitterman, E. L. Rickes, D. E. Wolf, J. Modas, S. B. Zimmerman, T. H. Stoudt, and T. C. Demny, J. Antibiot., 23, 305 (1970).

5. G. R. Pettit, R. B. Von Dreele, D. L. Herald, M. T. Edgar, and H. B. Wood, Jr., J. Am. Chem. Soc., 98, 6742 (1976).

6. G. S. Tarnowski, F. A. Schmid, D. J. Hutichison, and C. C. Stock, Cancer Chemother. Rep., 57, 21 (1973).

7. The name cyclo-(streptolutyl-streptolutyl) was proposed for 593A by Petit[5] to emphasize the relationship to the monomeric amino acid.

8. K. E. Harding, proposal submitted to National Institutes of Health.

9. C. Sellei, S. Eckhardt, and L. Nemeth, Chemotherapy of Neoplastic Disease, Akademiai Kaido, Budapest, 1970, pp. 128-177; A. Albert, Selective Toxicity, Sixth Edition, Chapman and Hall, London, 1979, pp. 464-469.

10. G. E. Foley, R. E. McCarthy, V. M. Binns, E. E. Snell, B. M. Guiraed, G. W. Kidder, V. C. Dewey, P. S. Thayer, Ann. N.Y. Acad. Sci., 76, 413 (1958).

11. V. R. Mandapur, Ind. J. Chem., 18B, 75 (1979).

12. K. E. Harding, Louis N. Moreno, and V. Mark Nace, J. Org. Chem., in press.

13. D. Shapiro, H. Segal, H. M. Flowers, J. Am. Chem. Soc., 80, 1194 (1958).

14. H. C. Brown, P. J. Geoghegan, Jr., J. T. Kurek, G. J. Lynch, Organometal. Chem. Syn., 1, 7 (1970/1971).

15. T. Fukuyama, C.-L. J. Wang, Y. Kishi, J. Am. Chem. Soc., 101:1, 262 (1979); E. J. Corey, H. L. Pearle, I. Szekely, M. Ishiguro, Tet. Lett., 12, 1023 (1978); J. Fried, J. Barton, Proc. Nat. Acad. Sci. U.S., 74, 2199 (1977); N. A. Nelson, J. Am. Chem. Soc., 99, 7362 (1977).

16. K. E. Harding and S. R. Burks, J. Org. Chem., submitted for publication.

17. R. A. Johnson, F. H. Lincoln, J. L. Thompson, E. G. Nidy, S. A. Mizak, U. Axen, J. Am. Chem. Soc., 99, 4182 (1977).