SPECIFICATION

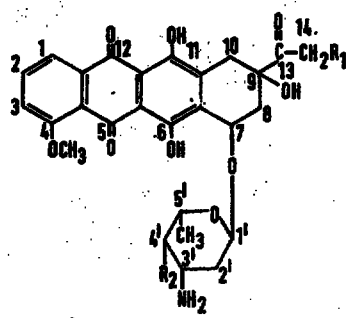
# ANTITUMOR ANTHRACYCLINE ANTIBIOTICS

This invention relates to anthracycline glycoside antibiotics, particularly for use as antimicrobial and antitumor agents, to a process for preparing them, and to pharmaceutical compositions containing them.

A number of anthracycline glycosides have been disclosed in the literature. Among them, daunomycin (U.S. Patent 3 616 242; U.K. Patent 1 003 383) and adriamycin (U.S. Patents 3 590 028 and 3 803 124), which are obtained from the cultured broth of certain streptomyces, have a broad antitumor spectrum against various experimental tumors and are used clinically as potent chemotherapeutic agents. Despite the usefulness of adriamycin and daunomycin as clinical antitumor agents, it is known that they have severe side effects such as alopecia, leukopenia and cardiotoxicity.

The present inventors have extensively studied the chemical modification of daunomycin and adriamycin with the aim of preparing new derivatives of these glycosides having increased anticancer activity and reduced toxicity (side effects). By the present invention, they have succeeded in providing new tetrahydropyranyl ether derivatives of adriamycin and daunomycin which possess the desired strong antitumor activity and low toxicity.

This invention relates to certain novel anthracycline glycoside derivatives having the general structural formula



I

wherein $R_1$ represents a hydrogen atom, a hydroxyl group or a tetrahydropyranyloxy group and $R_2$ represents a hydroxyl group or a tetrahydropyranyloxy group, providing that when $R_1$ is a hydrogen atom or hydroxyl group, $R_2$ is a tetrahydropyranyloxy group, and the nontoxic acid addition salts thereof. The compounds included within the scope of formula I exhibit both antimicrobial and antitumor activity.

As used herein and in the claims the term "nontoxic acid addition salt" is meant to include all those organic and inorganic acid salts of the compounds of formula I, which salts are commonly used as substantially nontoxic salts of medicinal agents containing amine functions. Illustrative examples would be those salts formed from such acids as sulfuric, phosphoric, hydrochloric, hydrobromic, nitric, phosphorous, acetic, propionic, maleic, oleic, palmitic, citric, succinic, tartaric, fumaric, glutamic, pantothenic, laurylsulfonic, methanesulfonic, naphthalenesulfonic.

The compounds represented by formula I having a tetrahydropyranyloxy $R_2$ substituent exist as the individual diastereomers (arbitrarily designated herein as isomer a and isomer b) which differ in the configuration at the C—2 position of the tetrahydropyranyloxy group or as mixtures of such isomers. The present invention includes the separate diastereomers as well as the diastereomeric mixtures within its scope.

In the accompanying drawings:

FIG. 1 shows the infrared absorption spectrum (KBr) of 4'-O-tetrahydropyranyl daunomycin (isomer a);

FIG. 2 shows the infrared absorption spectrum (KBr) of 4'-O-tetrahydropyranyl daunomycin (isomer b);

FIG. 3 shows the infrared absorption spectrum (KBr) of 4',14-bis(O-tetrahydropyranyl)adriamycin (isomer a);

FIG. 4 shows the infrared absorption spectrum (KBr) of 4',14-bis(O-tetrahydropyranyl)adriamycin (isomer b);

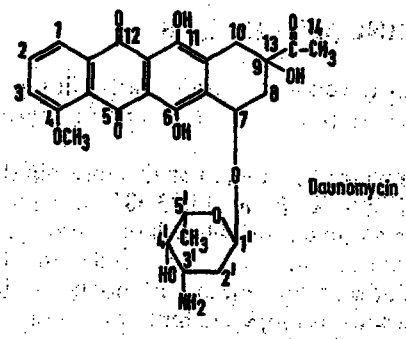
FIG. 5 shows the infrared absorption spectrum (KBr) or 14-O-tetrahydropyranyl adriamycin;

FIG. 6 shows the infrared absorption spectrum (KBr) of 4'-O-tetrahydropyranyl adriamycin (isomer a);

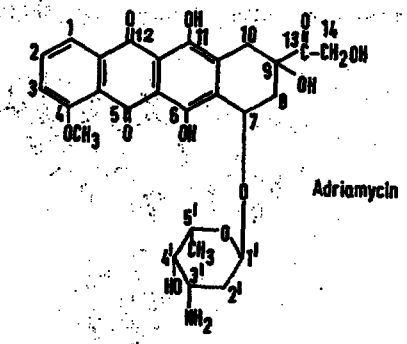
FIG. 7 shows the infrared absorption spectrum (KBr) of 4'-O-tetrahydropyranyl adriamycin (isomer b);

FIGS. 8 to 14 show proton NMR spectra (100 MHz, $CDCl_3$) of the compounds shown in the order of their infrared absorption spectra above.

Adriamycin and daunomycin, the starting materials for preparing the compounds of the present invention, may be represented by the formulae




and

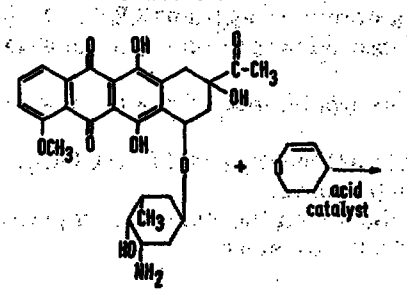



Daunomycin possesses two reactive hydroxyl groups (excluding the two phenolic hydroxyl groups) at C—9 and C—4′ and adriamycin has three reactive hydroxyl groups (again excluding the two phenolic groups) at C—9, C—14 and C—4′. The present inventors have discovered that under proper conditions there are differences in the reactivity of the various reactive hydroxyl groups in these compounds and that these differences can be utilized to prepare desirable new derivatives. More specifically, they have found that when the free base of adriamycin or daunomycin or an acid addition salt thereof (e.g. the hydrochloride) is suspended or dissolved in an inert organic solvent and reacted with 3,4-dihydro-2H-pyrane in the presence of an acid catalyst, various new tetrahydropyranyl ether derivatives of the starting material glycosides are formed. The particular reaction products formed, the ratios of the different products and the reaction yields vary with the reaction conditions used, e.g. solvent, acid catalyst, ratio of reactants, temperature, reaction time, etc.

The present invention thus provides a process for preparing an anthracycline glycoside of the general formula I, or a nontoxic acid addition salt thereof, which process comprises reacting adriamycin or daunomycin or an acid addition salt thereof with 3,4-dihydro-2H-pyrane in an inert organic solvent and in the presence of an acid catalyst to convert one or two of the hydroxyl groups at C—4′ or C—14 to tetrahydropyranyloxy groups and, if desired, selectively converting the C—14 tetrahydropyranyloxy group of a di-tetrahydropyranyl product to a hydroxyl group by alcoholysis or hydrolysis and, if desired, carrying out one or more of the further steps selected from (a) converting by methods known *per se* a product in the form of the free base or acid addition salt thereof to a nontoxic acid addition salt thereof or (b) converting by methods known *per se* a product in the form of an acid addition salt to the corresponding free base.

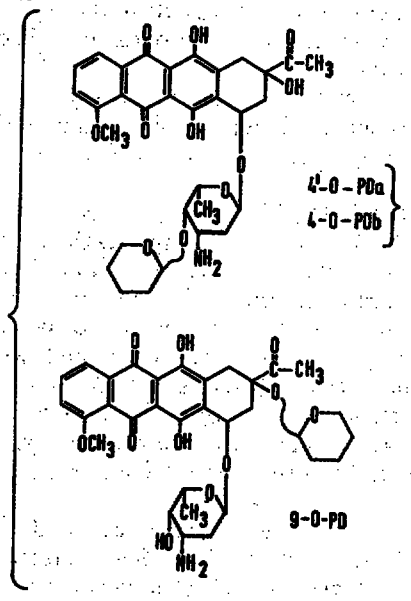
The general reaction processes for preparing the compounds of formula I may be more easily seen from the following schemes:

Scheme I

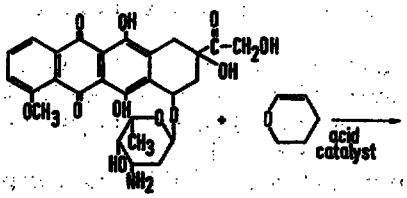


Scheme I continued

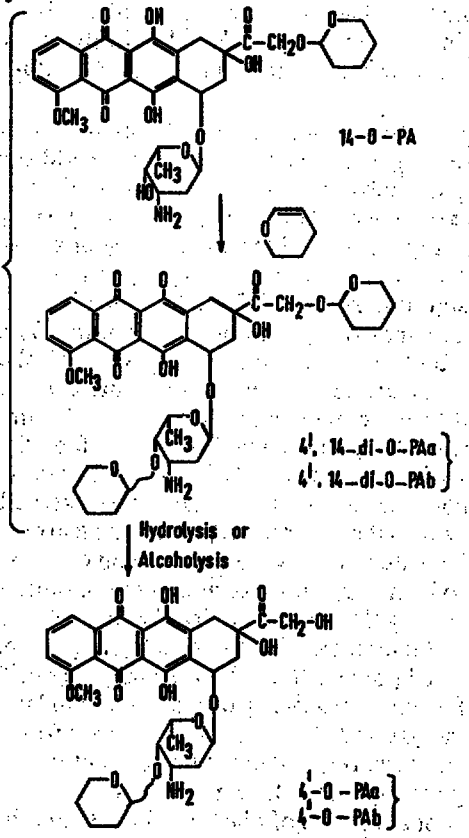
Daunomycin




Scheme II




Adriamycin



Conversion of a reactive hydroxyl group of adriamycin or daunomycin to a tetrahydropyranyloxy group is accomplished by etherification.

The starting material glycoside can be in free base form or in the form of an acid addition salt. Since acid addition salts of the tetrahydropyranyl ether products of the reaction may be converted by known methods to the corresponding free base products or to nontoxic acid addition salts, it is not necessary for a starting material salt to be nontoxic.

Any nonreactive organic solvent may be used for the tetrahydropyranylation reaction. Examples of suitable solvents include benzene, toluene, xylene, dimethylformamide, acetonitrile and tetrahydrofuran. The reaction solvent can be a single solvent or mixture of solvents. A most preferred solvent is anhydrous dimethylformamide.

The acid catalyst may be any organic (e.g. formic, trifluoroacetic) or inorganic (e.g. hydrochloric, phosphoric) acid. A preferred class of acid catalysts comprises the organic sulfonic acids. More preferred catalysts are the aromatic sulfonic acids such as p-toluenesulfonic acid and benzenesulfonic acid. A most preferred catalyst is p-toluenesulfonic acid.

The reaction temperature is not critical. Good results in the etherification reaction have been achieved at room temperature, although temperatures higher and lower than this may also be used.

Reaction time will vary with the particular process conditions selected, e.g. temperature, catalyst, solvent, etc. Selection of optimum reaction time to produce a specific product or mixture of products may be made by routine experimentation using the thin layer assay described below. In general, however, reaction times of from about 20 hours to about 50 hours have been found to give advantageous results.

While as mentioned above the particular reaction products and reaction yield are dependent on such factors as the concentration of starting materials, ratio of reactants, etc., when using daunomycin as a starting material the principal products are 4'-O-tetrahydropyranyl daunomycin (abbreviated as 4'-O-PD) and 9-O-tetrahydropyranyl daunomycin (abbreviated as 9-O-PD). These products may be detected in the reaction mixture by silica gel thin-layer chromatography (Merck Co. $60F_{254}$) using a mixture of chloroform-methanol-acetic acid (80:20:4 v/v) as a developer. The products appear at $R_f$ 0.74 (4'-O-PD) and $R_f$ 0.15 (9-O-PD).

The product 4'-O-PD was separated into two components having $R_f$ values of 0.46 and 0.65 on silica gel TLC chromatography using a mixture of chloroform-methanol (10:1 v/v). These components were found to be diastereomers of 4'-O-PD. Components $R_f$ 0.46 and $R_f$ 0.65 were arbitrarily designated as 4'-O-PDa (isomer a) and 4'-O-PDb (isomer b), respectively.

When using adriamycin as the starting material glycoside, the main products detected using the abovementioned silica gel TLC procedure were 14-O-tetrahydropyranyl adriamycin (14-O-PA) having $R_f = 0.12$ and two components which are diastereomers of 4',14-di(O-tetrahydropyranyl)adriamycin, i.e. 4',14-di(O-tetrahydropyranyl)adriamycin (isomer a), abbreviated 4',14-di-O-PAa at $R_f$ 0.55 and 4',14-di(O-tetrahydropyranyl)adriamycin (isomer b), abbreviated 4',14-di-O-PAb at $R_f$ 0.73. The compound 4',14-di(O-tetrahydropyranyl)adriamycin may also be named 4',14-bis(O-tetrahydropyranyl)adriamycin.

The diastereomeric mixture of 4',14-di-O-PAa and 4',14-di-O-PAb may also be prepared in high yield by etherifying 14-O-tetrahydropyranyl adriamycin or an acid addition salt thereof with 3,4-dihydro-2H-pyrane in an inert organic solvent and in the presence of an acid catalyst.

By utilizing the difference in reactivity between the C—14 primary hydroxyl group and the C—4' secondary hydroxyl group, the tetrahydropyranyl group at C—14 of 4',14-di-O-PAa and 4',14-di-O-PAb (or an acid addition salt thereof) can be selectively removed by hydrolysis or alcoholysis to produce in good yield the corresponding diastereomers of 4'-O-PAa and 4'-O-PAb. Conversion of the tetrahydropyranyloxy group to a hydroxy group may be carried out, for example by hydrolysis with acidified water (i.e. aqueous inorganic or organic acid) or by alcoholysis with an alcohol or phenol (e.g. a $C_1$—$C_6$ alkanol). A convenient procedure comprises treatment with dilute acetic acid solution or p-toluenesulfonic acid-methanol solution at room temperature for a period of from about 30 minutes to 5 hours. Side reaction can be minimized by carrying out the hydrolysis or alcoholysis reaction in the dark.

The products of formula I may be isolated from the reaction mixture by conventional procedures. Thus, products of the tetrahydropyranylation reaction may be recovered by neutralizing the reaction mixture with a basic substance (e.g. an alkali metal carbonate or bicarbonate), extracting the neutralized reaction mixture with a water-immiscible organic solvent (e.g. ethyl acetate, chloroform, methylene chloride, methyl isobutyl ketone, etc.), extracting the organic extract with dilute aqueous acid (organic or inorganic), neutralizing the aqueous acidic layer with a basic substance, extracting the neutralized aqueous layer with a water-immiscible organic solvent and concentrating the organic extract to dryness. The dark-red dried powder thus obtained may be purified by silica gel column chromatography or, in the case of a small sample, by preparative thin layer chromatography. The products of the hydrolysis or alcoholysis reaction may be recovered from the reaction mixture by neutralizing with a basic substance, extracting the neutralized reaction mixture with a water-immiscible organic solvent and concentrating the organic extract to dryness.

Products obtained in the form of a mixture of diastereomers (a and b isomers) may be separated as

described above by silica gel thin layer chromatography into the individual a and b isomers in substantially pure form.

Products obtained in the above reaction procedures may be recovered in the form of the free base, an acid addition salt or a nontoxic acid addition salt. The free base products may be easily converted into nontoxic acid addition salts which are substantially equivalent in therapeutic activity to the corresponding free bases. The salts are formed, isolated, purified and formulated by the methods generally employed in salt formation for antibiotics, e.g. the anthracycline glycoside antibiotics. Thus, the free base may be reacted with a nontoxic acid in a suitable solvent and the salt recovered by lyophilization or by precipitation with an antisolvent, i.e. a solvent in which the desired salt is only slightly soluble. Products in the form of an acid addition salt may be converted to the corresponding free base by neutralization with a basic substance. Finally, toxic acid addition salts may be converted to nontoxic acid addition salts by neutralization and treatment with a nontoxic acid as described above.

Physicochemical Properties

The compounds of the present invention exist in solid form as an amorphous or crystalline red powder. As free bases they are soluble in ethyl acetate, chloroform and ethanol and slightly soluble in water, n-hexane, petroleum ether, etc. Ethanol solutions and acidic solutions of the compounds are of a red colour, give a positive ninhydrin reaction and do not reduce Fehling's solution. Figures 1—14 and Table 1 show the elemental analysis, melting point (decomposition), specific rotation ($C = 0.2$ in $CHCl_3$), UV and visible absorption spectrum (methanol), infrared absorption spectrum (KBr tablet) and nuclear magnetic resonance spectrum (100 MHz, $CDCl_3$).

TABLE 1

Physicochemical Properties of Pyranyl Derivatives

| Compounds | 4′-O-PDa | 4′-O-PDb | 4′-14-di-O-PAa | 4′,14-di-O-PAB |
|---|---|---|---|---|
| Analytical data | | | | |
| (1) Elementary analysis | C: 60.82 (61.04) | C: 61.48 (61.04) | C: 61.12 (60.89) | C: 61.25 (60.89) |
| | H: 6.27 ( 6.24) | H: 6.37 (6.24) | H: 6.75 ( 6.50) | H: 6.81 ( 6.50) |
| ( ) Calcd. %* | N: 2.24 ( 2.22) | N: 1.97 (2.22) | N: 1.86 (1.92) | N: 1.84 ( 1.92) |
| (2) Molecular weight | 611.70 | 611.70 | 711.83 | 711.83 |
| (3) Melting point (°C) | 193 ~ 196 (decomp.) | 190 ~ 193 (decomp.) | 180 ~ 186 (decomp.) | 178 ~ 182 (decomp.) |
| (4) Specific rotation C = 0.2 $CHCl_3$ | $[\alpha]_D^{22}$ + 125° | $[\alpha]_D^{22}$ + 162.5° | $[\alpha]_D^{24}$ + 25° | $[\alpha]_D^{24}$ + 125° |
| (5) $R_f$ value** | 0.46 | 0.65 | 0.55 | 0.73 |
| (6) UV visible absorption spectrum (nm) $\left(E_{1cm}^{1\%}\right)$ | 222s(335), 234(515)<br>252.5(350), 289(120)<br>480(140), 496(140)<br>532(80), 576(15) | 222s(330), 234.5(485)<br>252.5(350), 290(115)<br>480(135), 498(140)<br>532(85), 576(20) | 220s(320), 234.5(480)<br>253(360), 290(110)<br>480s(145), 498(155)<br>532(110), 577(40) | 220s(310), 234.5(460)<br>253(350), 290(105)<br>480s(140), 497(150)<br>532(105), 576(40) |

*Calculated as monohydrate

**Silica gel TLC: $CHCl_3$:$CH_3OH$ = 10:1 (v/v), 26°C (Merck Co. $60F_{254}$)

TABLE 1 (Continued)

| Compounds | 14-O-PA | 4'-O-PAa | 4'-O-PAb |
|---|---|---|---|
| Analytical data | | | |
| (1) Elementary analysis | C: 59.71 (59.52) | C: 59.65 (59.52) | C: 59.71 (59.52) |
| | H: 6.23 ( 6.10) | H: 6.33 ( 6.10) | H: 6.24 ( 6.10) |
| ( ) Calcd. %* | N: 2.33 ( 2.17) | N: 2.21 ( 2.17) | N: 2.05 ( 2.17) |
| (2) Molecular weight | 627.70 | 627.70 | 627.70 |
| (3) Melting point (°C) | 195 ~ 202 (decomp.) | 172 ~ 177 (decomp.) | 188 ~ 192 (decomp.) |
| (4) Specific rotation $c = 0.2$ $CHCl_3$ | $[\alpha]_D^{24}$ + 162.5° | $[\alpha]_D^{26}$ + 150° | $[\alpha]_D^{25}$ + 150° |
| (5) $R_f$ value** | 0.12 | 0.32 | 0.49 |
| (6) UV visible absorption spectrum (nm) $E_{1cm}^{1\%}$ | 220s(340), 234(530)<br>252.5(400), 290(115)<br>480s(160), 497(170)<br>532(135), 577(65) | 220s(350), 234(515)<br>252.5(360), 290(120)<br>480(150), 497(160)<br>532(100), 577(30) | 220s(365), 234(480)<br>252(350), 290(110)<br>480s(135), 498(140)<br>531.5(100), 580(45) |

*Calculated as monohydrate

**Silica gel TLC: $CHCl_3$:$CH_3OH$ = 10:1 (v/v), 26°C (Merck Co. $60F_{254}$)

With respect to the structure of the compounds 4'-O-PDa, 4'-O-PDb, 4',14-di-O-PAa, 4',14-di-O-PAb, 14-O-PA, 4'-O-PAa and 4'-O-PAb of the present invention, the number of tetrahydropyranyl groups bound to the compounds can be analyzed to be either one or two by the signal intensity of methine proton at C—2 and the methylene proton at the C—3, C—4, C—5 and C—6 positions of the tetrahydropyranyl group. The binding position of the tetrahydropyranyl group can be analyzed by the chemical shift of the C—4' proton in the daunosamine moiety towards a lower field (compared with that of daunomycin) due to the formation of the glycosidic bond at C—4'.

The difference in configuration between 4'-O-PDa and 4'-O-PDb, 4'-O-PAa and 4'-O-PAb and 4',14-di-O-PAa and 4',14-pi-O-PAb is considered to be the difference in absolute configuration R and S at C—2 of the tetrahydropyranyl group since the chemical shifts and coupling constants (J value) at the C—2 and C—3 protons differ from each other. The absolute configuration, however, for the a and b isomers is still unknown. Table 2 shows the chemical shifts (from FIGS. 8—14) at C—2 of the tetrahydropyranyl group and at the C—4' proton of the daunosamine moiety.

TABLE 2

| Proton | 4'-THP* (ppm) | 14-THP* (ppm) | DS4'** (ppm) |
|---|---|---|---|
| Compound | | | |
| 4'-O-PDa | 4.38 | — | 3.62 |
| 4'-O-PDb | 4.72 | — | 3.64 |
| 4'-O-PAa | 4.36 | — | 3.70 |
| 4'-O-PAb | 4.72 | — | 3.70 |
| 4',14-di-O-PAa | 4.38 | 4.70 | 3.60 |
| 4',14-di-O-PAb | 4.72 | 4.72 | 3.66 |
| 14-O-PA | — | 4.71 | 3.48 |
| Daunomycin | — | — | 3.49 |

*THP: Chemical shift at C—2 methine of substituted tetrahydropyranyl group

**DS4': Chemical shift at C—4' methine of daunosamine

From the above, the structures of the compounds of the present invention were determined to be as indicated above.

Antibiotic Activity

The compounds of formula I have been found to possess antimicrobial activity against a variety of pathogenic microorganisms. The minimum inhibitory concentrations (as determined by the broth dilution method) of representative compounds of the present invention are shown in Table 3.

TABLE 3

Minimum Inhibitory Concentration (MIC, mcg./ml.)

| Test Organisms | Compound Tested | | | |
|---|---|---|---|---|
| | 4'-O-PDa | 4'-O-PDb | 4'-O-PAa | 4'-O-PAb |
| *Staph. aureus* FDA 209P | 6.25 | 6.25 | 6.25 | 6.25 |
| *Staph. aureus* Smith | 12.5 | 3.12 | 6.25 | 6.25 |
| *Bacillus subtilis* NRRLB-558 | 3.12 | 1.56 | 3.12 | 3.12 |
| *Bacillus cereus* ATCC 10702 | 6.25 | 6.25 | 6.25 | 6.25 |
| *Bacillus megaterium* APF | 6.25 | 3.12 | 3.12 | 3.12 |
| *Sarcina lutea* PCI 1001 | 0.39 | 0.39 | 0.78 | 0.78 |
| *Micrococcus flavus* FDA 16 | 0.78 | 1.56 | 3.12 | 3.12 |
| *Corynebacterium bovis* 1810 | 0.78 | 0.78 | 3.12 | 3.12 |
| *Pseudomonas aeruginosa* A3 | >100 | >50 | >100 | >100 |
| *Escherichia coli* NIHJ | >100 | >100 | >100 | >100 |
| *Mycobacterium smegmatis* ATCC 607 | 6.25 | 6.25 | 100 | 100 |
| *Candida albicans* | >50 | 25 | >100 | >50 |

As indicated by Table 3, the present compounds are useful an antibiotic agents, particularly against gram-positive bacteria.

Antitumor Activity

The compounds of formula I show marked antitumor activity with low toxicity in standard tests.

A. The present compounds were found to have a marked inhibitory effect on the growth and nucleic acid synthesis of L1210 leukemia cells in culture.

For example, L1210 cells ($5 \times 10^4$ cells/ml.) were inoculated in RPMI 1640 medium (Roswell Park Memorial Institute 1640) containing 20% calf serum and cultivated at 37°C in the presence of 0.1 and 0.5 μg./ml. of the compounds of the present invention in a $CO_2$ incubator. The number of cells were periodically counted and the growth inhibition rate (%) of control was determined as shown in Table 4.

TABLE 4

Growth Inhibitory Effect of Tetrahydropyranyl Derivatives on L1210 Cells in Culture

| Concentration | Inhibition Rate (%) | |
|---|---|---|
| Compounds | 0.1 | 0.5 μg./ml. |
| 4'-O-PDa | 79.2 | 95.0 |
| 4'-O-PDb | 74.6 | 88.8 |
| Daunomycin | 68.8 | 72.7 |
| 4'-O-PAa | 65.9 | 81.1 |
| 4'-O-PAb | 78.1 | 92.2 |
| 14-O-PA | 7.6 | 58.3 |
| 4';14-di-O-PAa | 37.5 | 80.8 |
| 4';14-di-O-PAb | 25.5 | 72.1 |
| Adriamycin | 70.7 | 84.2 |

The effect of the compounds of the present invention on nucleic acid synthesis was examined as follows:

$1 \times 10^5$ cells/ml. of L1210 cells were suspended in RPMI medium containing 10% calf serum, pre-cultivated at 37°C for 1 to 2 hours in a $CO_2$ incubator and then the compounds of the present invention were added to the medium at various concentrations. After 15 min. of incubation, $^{14}$C-uridine (0.05 μCi/ml.) or $^{14}$C-thymidine (0.05 μCi/ml.) was added and incubated at 37°C for 60 min. Trichloroacetic acid (TCA) (10%) was added to the incubation medium to stop the reaction and precipitate the acid-insoluble materials, and then the precipitate was washed three times with 5 to 10% TCA dissolved in formic acid. The radioactivity was measured and expressed as 50% inhibition concentration of incorporation.

TABLE 5

50% Inhibition Concentration of $^{14}$C-Thymidine and $^{14}$C-Uridine Incorporation into L1210 Cells in Culture

| Compounds | 50% Inhibition Concentration μg./ml. | |
|---|---|---|
| | Uridine | Thymidine |
| 4'-O-PDa | 0.13 | 0.28 |
| 4'-O-PDb | 0.20 | 0.32 |
| Daunomycin | 0.40 | 0.70 |
| 4'-O-PAa | 0.20 | 0.37 |
| 4'-O-PAb | 0.24 | 0.50 |
| 14-O-PA | 0.17 | 0.42 |
| 4';14-di-O-PAa | 0.23 | 0.55 |
| 4';14-di-O-PAb | 0.24 | 0.97 |
| Adriamycin | 0.50 | 2.1 |

B. When tested against various experimental animal tumors, the present compounds show a marked antitumor activity with reduced toxicity relative to adriamycin and daunomycin. Accordingly, the compounds are therapeutically useful in inhibiting the growth of mammalian tumors.

As an example, $BDF_1$ mice were inoculated intraperitoneally with $1 \times 10^6$ cells/mouse of L1210 leukemia cells. After 24 hours had elapsed since inoculation, the mice were administered the compounds of the present invention intraperitoneally once daily for ten consecutive days and observed for a 45 day period. The antitumor activity was shown by the prolongation rate of survival day (T/C, %) to the survival day of control mice injected with physiological saline. The results are shown in Table 6.

TABLE 6

Antitumor Activity of Tetrahydropyranyl Derivatives (T/C, %)

| Compounds | Dose (mg./kg./day) | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 2.5 | 1.25 | 0.6 | 0.3 | 0.15 |
| 4'-O-PDa | >320 | >320 | 122 | 115 | 96 | 90 |
| 4'-O-PDb | >320 | 256 | 122 | 115 | 103 | 90 |
| 4'-O-PAa | – | 173 | 180 | 187 | 120 | 127 |
| 4'-O-PAb | >375 | >360 | >373 | 293 | 160 | 113 |
| 14-O-PA | 142 | 130 | 126 | 113 | 110 | 103 |
| 4',14-di-O-PAa | 154 | 115 | 109 | 96 | 103 | 96 |
| 4',14-di-O-PAb | 161 | 109 | 103 | 103 | 96 | 115 |
| Adriamycin | toxic death | 231 | 218 | 230 | 165 | 128 |

From the results of toxic death and body weight loss of the mice in this experiment, it is shown that the derivatives of the present invention are 1/3 to 1/2 lower in toxicity than adriamycin and daunomycin which are the starting materials in the present invention.

C. The marked antitumor effects seen from A and B above were confirmed by the stability of the present compounds on the inactivation by hepatic NADPH-cytochrome P450 reductase. Specifically, NADPH-cytochrome P450 reductase purified from rat liver homogenate was incubated with the compounds of the present invention at 25°C for 25 min. in nitrogen gas phase, and the incubation product formed, 7-deoxyaglycone, was determined as shown in Table 7.

TABLE 7

Stability of Tetrahydropyranyl Derivatives on Rat NADPH Cytochrome P450 Reductase

| Compounds | Product (nmoles/tube) (7-Deoxyaglycone) |
|---|---|
| 4'-O-PDa | 37.3 |
| 4'-O-PDb | 46.6 |
| Daunomycin | 65.8 |
| 4'-O-PAa | 10.9 |
| 4'-O-PAb | 15.8 |
| 14-O-PA | 18.4 |
| 4',14-di-O-PAa | 13.1 |
| 4',14-di-O-PAb | 15.8 |
| Adriamycin | 47.2 |

Composition of reaction mixture:

| | |
|---|---|
| NADPH | 0.2 mM |
| Tris-HCl (pH 8.0) | 0.1 M |
| Substrate | 0.1 mM |
| Enzyme | 4.6 μg./ml |

(Tris-HCl=Tris (hydroxymethyl) amino methane)

Therapeutic Use

As mentioned above, the compounds 4'-O-PDa, 4'-O-PDb, 4',14-di-O-PAa, 4',14-di-O-PAb, 14-O-PA, 4'-O-PAa and 4'-O-PAb and their nontoxic acid addition salts are novel antibiotics, useful in both human and veterinary medicine, and also possess marked inhibitory action against malignant mammalian tumors, including both solid and ascitic types.

According to one aspect of the invention, a method is provided for therapeutically treating a mammalian host affected by a microbial infection (particularly a gram-positive bacterial infection) or by a malignant tumor (i.e. a solid- or ascitic-type tumor such as L1210 leukemia) which comprises administering to said host an effective antimicrobial or tumor-inhibiting dose of 4'-O-PDa, 4'-O-PDb, 4',14-di-O-PAa, 4',14-di-O-PAb, 14-O-PA, 4'-O-PAa or 4'-O-PAb, or a nontoxic acid addition salt thereof, or a mixture thereof.

According to another aspect of the invention, a pharmaceutical composition is provided which comprises a therapeutically effective antimicrobial or tumor-inhibiting amount of 4'-O-PDa, 4'-O-PDb, 4',14-di-O-PAa, 4',14-di-O-PAb, 14-O-PA, 4'-O-PAa or 4'-O-PAb, or a mixture thereof, or a nontoxic acid addition salt thereof, in combination with a pharmaceutical carrier or diluent. Such compositions may be made up in any pharmaceutical form appropriate for parenteral administration.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions or emulsions. They may also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, physiological saline or some other sterile injectable medium immediately before use.

It will be appreciated that the actual preferred dosage amounts used will vary according to the particular compound being used, the particular composition formulated, the mode of application and the particular situs, host and disease being treated. In general the compounds are injected intraperitoneally, intravenously, subcutaneously or locally into non-human mammals and intravenously or locally into humans. Many factors that modify the action of the drug will be taken into account by those skilled in the art, for example, age, body weight, sex, diet, time of administration, route of administration, rate of excretion, condition of the patient, drug combinations, reaction sensitivities and

severity of the disease. Administration can be carried out continuously or periodically within the maximum tolerated dose. Optimal application rates for a given set of conditions can be ascertained by those skilled in the art using conventional dosage determination tests in view of the above guidelines.

For use as an antimicrobial agent, the compounds are in general administered so that the concentration of active ingredient is greater than the minimum inhibitory concentration for the particular organism being treated.

The following examples are provided for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1

Process for Producing 4'-O-Tetrahydropyranyl Daunomycin (4'-O-PDa and 4'-O-PDb)

To a solution of daunomycin hydrochloride (60 mg.) in 5 ml. of anhydrous dimethylformamide was added 1 ml. of 3,4-dihydro-2H-pyrane and a catalytic amount of p-toluenesulfonic acid. After being allowed to stand overnight at room temperature in the dark, the reaction mixture was added to 20 ml. of 0.1 N sodium hydrogen carbonate aqueous solution and extracted with chloroform (10 ml. × 4). After the chloroform-extract was extracted with 1% acetic acid solution (10 ml. × 10), the resulting acidic aqueous layer was neutralized with sodium hydrogen carbonate and then re-extracted with chloroform (20 ml. × 10). The chloroform layer was dried over anhydrous sodium sulfate and concentrated to dryness. The 44 mg. of residue thus obtained was applied to preparative silica gel thin-layer chromatography (Merck Co. $60F_{254}$) and developed by a chloroform-methanol mixture (10:1) (v/v).

Silica gel bands corresponding to $R_f$ 0.46 and 0.65 were scratched out from the thin-layer, eluted with the chloroform-methanol mixture (10:1) (v/v) and concentrated to dryness. Each residue was dissolved in methylene chloride, frozen by addition of *t*-butanol under cooling and dried under reduced pressure. There were obtained 10.1 mg. of reddish brown solid of 4'-O-PDa and 10.3 mg. of red solid of 4'-O-PDb from the $R_f$ 0.46 and 0.65 fractions, respectively.

4'-O-PDa and 4'-O-PDb are diastereomers of 4'-O-tetrahydropyranyl daunomycin, and their physicochemical properties are as shown in Table 1.

EXAMPLE 2

Process for Producing 4',14-O-bis(Tetrahydropyranyl)adriamycin (4',14-di-O-PAa and 4',14-di-O-PAb) and 14-O-Tetrahydropyranyl Adriamycin (14-O-PA) from Adriamycin

To a solution of adriamycin hydrochloride (130 mg.) in 10 ml. of anhydrous dimethylformamide was added 2 ml. of 3,4-dihydro-2H-pyrane and a catalytic amount of p-toluenesulfonic acid. After being allowed to stand for 48 hours at room temperature in the dark, the reaction mixture was added to 20 ml. of 0.1N sodium hydrogen carbonate aqueous solution and extracted with ethyl acetate (20 ml. × 5). After extracting the ethyl acetate layer with 1% acetic acid solution (40 ml. × 4), the acidic aqueous layer was neutralized with sodium hydrogen carbonate and extracted with chloroform (20 ml. × 10). The chloroform layer was dried over anhydrous sodium sulfate and concentrated to dryness. The 160 mg. of resulting solid was developed and purified by a preparative silica gel thin-layer chromatography using a chloroform-methanol mixture (10:1) (v/v). The bands of $R_f$ 0.12 and 0.55 were scratched out from the thin-layer and purified according to the method of Example 1.

There were obtained 35 mg. of red solid of 14-O-PA, 16 mg. of red solid of 4',14-di-O-PAa and 14 mg. of red solid of 4',14-di-O-PAb from the $R_f$ 0.12, 0.55 and 0.73 fractions, respectively.

4',14-di-O-PAa and 4',14-di-O-PAb are diastereomers of 4',14-bis(O-tetrahydropyranyl) adriamycin and the physicochemical properties are as shown in Table 1.

EXAMPLE 3

Process for Producing 4',14-bis(O-Tetrahydropyranyl)-adriamycin from 14-O-Tetrahydropyranyl Adriamycin

To a solution of 35 mg. of 14-O-PA in 2 ml. of anhydrous dimethylformamide, there was added 0.5 ml. of 3,4-dihydro-2H-pyrane and a catalytic amount of p-toluenesulfonic acid. After being allowed to stand for 40 hours at room temperature in the dark, the reaction mixture was added to 10 ml. of 0.02N sodium hydrogen carbonate aqueous solution and extracted with ethyl acetate (5 ml. × 4). After extracting the ethyl acetate layer with 1% acetic acid solution (10 ml. × 3), the acidic aqueous layer was neutralized with sodium hydrogen carbonate and extracted with chloroform (10 ml. × 5). The chloroform layer was dried over anhydrous sodium sulfate and concentrated to dryness.

The resulting residue was chromatographed using a silica gel thin-layer according to Example 2, and 8.4 mg. of red solid of 4',14-di-O-PAa and 8.1 mg. of red solid of 4',14-di-O-PAb were obtained from the $R_f$ 0.55 and 0.73 fractions, respectively. Their physicochemical properties coincided with those of the compounds obtained in Example 2.

EXAMPLE 4

Process for Producing 4'-O-Tetrahydropyranyl Adriamycin from 4',14-bis(O-Tetrahydropyranyl)adriamycin

a) 4',14-di-O-PAa (12.4 mg.) was dissolved in 1.5 ml. of 10% acetic acid solution and allowed to

stand for 4.5 hours at room temperature in the dark. The reaction mixture was added to 10 ml. of water, neutralized with sodium hydrogen carbonate powder, and extracted with chloroform (15 ml. × 2).

The chloroform layer was dried over anhydrous sodium sulfate and concentrated to dryness. The 11 mg. of resulting residue was purified by a silica gel thin-layer chromatography as described above using a chloroform-methanol mixture (10:1) (v/v). The main band at $R_f$ 0.32 was scratched out and eluted with the chloroform-methanol mixture (10:1) (v/v). The eluate was concentrated to dryness. The residue was dissolved in methylene chloride, adding *t*-butanol under cooling to freeze, and dried under reduced pressure. There was obtained 7 mg. of red solid of 4'-O-PAa and its physicochemical properties are shown in Table 1.

b) 4',14-di-O-PAb (16 mg.) was dissolved in 5 ml. of 0.005N p-toluenesulfonic acid-methanol solution and allowed to stand for 1 hour at room temperature in the dark. The reaction mixture was neutralized with 10 ml. of 0.01N sodium hydrogen carbonate aqueous solution and extracted with chloroform (10 ml. × 4). The chloroform layer was dried over anhydrous sodium sulfate and treated as shown in (a). There was obtained 7.2 mg. of red solid of 4'-O-PAb which shows $R_f$ 0.49 on a silica gel thin-layer under the conditions as described above. Its physicochemical properties are shown in Table 1.

4'-O-PAa and 4'-O-PAb are diastereomers of 4'-O-tetrahydropyranyl adriamycin.

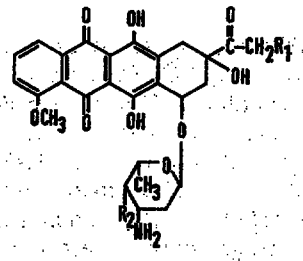
EXAMPLE 5

Salt Formation

Illustrative of the procedures which may be used to prepare acid addition salts, the free base of 4'-O-PDa, 4'-O-PDb, 4',14-di-O-PAa, 4',14-di-O-PAb, 14-O-PA, 4'-O-PAa or 4'-O-PAb may be dissolved in ethyl acetate and about one equivalent of HCl added. On lyophilization, the appropriate hydrochloride salt is obtained.

CLAIMS

1. An anthracycline glycoside of the general formula I:



(I)

wherein, $R_1$ represents a hydrogen atom or a hydroxyl or tetrahydropyranyloxy group, and $R_2$ represents a hydroxyl or tetrahydropyranyloxy group, providing that when $R_1$ is a hydrogen atom or a hydroxyl group, $R_2$ is a tetrahydropyranyloxy group, or a non-toxic acid addition salt thereof.

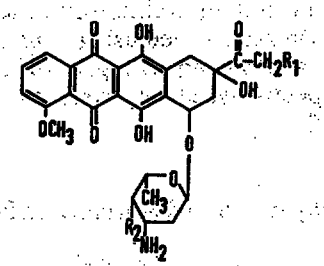
2. An anthracycline glycoside 4'-O-tetrahydropyranyl daunomycin (a) or 4'-O-tetrahydropyranyl daunomycin (b) according to claim 1 having the formula I, wherein, $R_1$ is a hydrogen atom, and $R_2$ is a tetrahydropyranyloxy group, or a non-toxic acid addition salt thereof.

3. An anthracycline glycoside 14-O-tetrahydropyranyl adriamycin according to claim 1 having the formula I, wherein, $R_1$ is a tetrahydropyranyloxy group, and $R_2$ is a hydroxyl group, or a non-toxic acid addition salt thereof.

4. An anthracycline glycoside 4',14-bis(O-tetrahydropyranyl) adriamycin (a) or 4',14-bis(O-tetrahydropyranyl) adriamycin (b) according to claim 1 having the formula I, wherein, $R_1$ and $R_2$ are a tetrahydropyranyloxy group, or a non-toxic acid addition salt thereof.

5. An anthracycline glycoside 4'-O-tetrahydropyranyl adriamycin (a) or 4'-O-tetrahydropyranyl adriamycin (b) according to claim 1 having the formula I, wherein, $R_1$ is a hydroxyl group, and $R_2$ is a tetrahydropyranyloxy group, or a non-toxic acid addition salt thereof.

6. A process for producing an anthracycline glycoside of the general formula I:



(I)

stand for 4.5 hours at room temperature in the dark. The reaction mixture was added to 10 ml. of water, neutralized with sodium hydrogen carbonate powder, and extracted with chloroform (15 ml. x 2).

The chloroform layer was dried over anhydrous sodium sulfate and concentrated to dryness. The 11 mg. of resulting residue was purified by a silica gel thin-layer chromatography as described above using a chloroform-methanol mixture (10:1) (v/v). The main band at $R_f$ 0.32 was scratched out and eluted with the chloroform-methanol mixture (10:1) (v/v). The eluate was concentrated to dryness. The residue was dissolved in methylene chloride, adding *t*-butanol under cooling to freeze, and dried under reduced pressure. There was obtained 7 mg. of red solid of 4'-O-PAa and its physicochemical properties are shown in Table 1.

b) 4',14-di-O-PAb (16 mg.) was dissolved in 5 ml. of 0.005N p-toluenesulfonic acid-methanol solution and allowed to stand for 1 hour at room temperature in the dark. The reaction mixture was neutralized with 10 ml. of 0.01N sodium hydrogen carbonate aqueous solution and extracted with chloroform (10 ml. x 4). The chloroform layer was dried over anhydrous sodium sulfate and treated as shown in (a). There was obtained 7.2 mg. of red solid of 4'-O-PAb which shows $R_f$ 0.49 on a silica gel thin-layer under the conditions as described above. Its physicochemical properties are shown in Table 1.

4'-O-PAa and 4'-O-PAb are diastereomers of 4'-O-tetrahydropyranyl adriamycin.

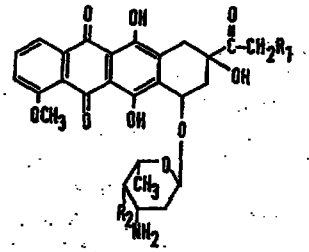
EXAMPLE 5

Salt Formation

Illustrative of the procedures which may be used to prepare acid addition salts, the free base of 4'-O-PDa, 4'-O-PDb, 4',14-di-O-PAa, 4',14-di-O-PAb, 14-O-PA, 4'-O-PAa or 4'-O-PAb may be dissolved in ethyl acetate and about one equivalent of HCl added. On lyophilization, the appropriate hydrochloride salt is obtained.

CLAIMS

1. An anthracycline glycoside of the general formula I:



(I)

wherein, $R_1$ represents a hydrogen atom or a hydroxyl or tetrahydropyranyloxy group, and $R_2$ represents a hydroxyl or tetrahydropyranyloxy group, providing that when $R_1$ is a hydrogen atom or a hydroxyl group, $R_2$ is a tetrahydropyranyloxy group, or a non-toxic acid addition salt thereof.

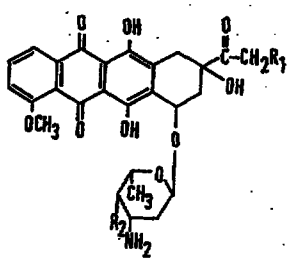
2. An anthracycline glycoside 4'-O-tetrahydropyranyl daunomycin (a) or 4'-O-tetrahydropyranyl daunomycin (b) according to claim 1 having the formula I, wherein, $R_1$ is a hydrogen atom, and $R_2$ is a tetrahydropyranyloxy group, or a non-toxic acid addition salt thereof.

3. An anthracycline glycoside 14-O-tetrahydropyranyl adriamycin according to claim 1 having the formula I, wherein, $R_1$ is a tetrahydropyranyloxy group, and $R_2$ is a hydroxyl group, or a non-toxic acid addition salt thereof.

4. An anthracycline glycoside 4',14-bis(O-tetrahydropyranyl) adriamycin (a) or 4',14-bis(O-tetrahydropyranyl) adriamycin (b) according to claim 1 having the formula I, wherein, $R_1$ and $R_2$ are a tetrahydropyranyloxy group, or a non-toxic acid addition salt thereof.

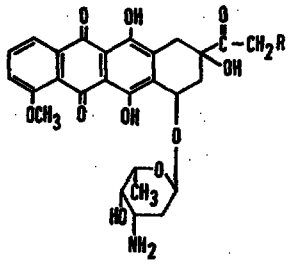
5. An anthracycline glycoside 4'-O-tetrahydropyranyl adriamycin (a) or 4'-O-tetrahydropyranyl adriamycin (b) according to claim 1 having the formula I, wherein, $R_1$ is a hydroxyl group, and $R_2$ is a tetrahydropyranyloxy group, or a non-toxic acid addition salt thereof.

6. A process for producing an anthracycline glycoside of the general formula I:



(I)

wherein, $R_1$ represents a hydrogen atom or a hydroxyl or tetrahydropyranyloxy group, and $R_2$ represents a hydroxyl or tetrahydropyranyloxy group, providing that when $R_1$ is a hydrogen atom or a hydroxyl group, $R_2$ is a tetrahydropyranyloxy group, or a non-toxic acid addition salt thereof, which comprises introducing one or two tetrahydropyranyl groups into hydroxyl groups at C—4′ or C—14 or both positions of an anthracycline glycoside of formula II:



(II)

wherein, R is a hydrogen atom or a hydroxyl group, or an acid addition salt thereof by reacting 3,4-dihydro-2H-pyrane with said glycoside or an acid addition salt thereof in an inert organic solvent and in the presence of an acid catalyst and optionally further comprises eliminating one tetrahydropyranyl group by partial hydrolysis or alcoholysis of the di-tetrahydropyranyl compound thus produced.

7. A process according to claim 6 which comprises introducing one or two tetrahydropyranyl groups into hydroxyl groups at C—14 or C—4′ and C—14 of the anthracycline glycoside of formula II, wherein, R is a hydroxyl group, or an acid addition salt thereof by reacting 3,4-dihydro-2H-pyrane with said glycoside of formula II or an acid addition salt thereof in an inert organic solvent and in the presence of an acid catalyst.

8. A process according to claim 6 which comprises introducing a tetrahydropyranyl group into the hydroxyl group at C—4′ of the anthracycline glycoside of formula II, wherein, R is a tetrahydropyranyloxy group, by reacting 3,4-dihydro-2H-pyrane with said glycoside or an acid addition salt thereof in an inert organic solvent and in the presence of an acid catalyst.

9. A process according to claim 6 which comprises hydrolyzing or alcoholizing the di-substituted compound having tetrahydropyranyl groups at C—14 and C—4′ of the anthracycline glycoside of formula II, wherein, R is a hydroxyl group, or an acid addition salt thereof to selectively convert it to a 4′-substituted compound.

10. A process according to claim 6 which comprises introducing a tetrahydropyranyl group into the hydroxyl group at C—4′ of the anthracycline glycoside of formula II, wherein, R is a hydrogen atom, or an acid addition salt thereof, by reacting 3,4-dihydro-2H-pyrane with said glycoside or an acid addition salt thereof in an inert organic solvent and in the presence of acid catalyst.

11. A process according to any of claims 6 to 10 which includes the step of converting a product in the form of a free base to a non-toxic acid addition salt thereof.

12. A process according to any of claims 6 to 10 which includes the step of converting a product in the form of an acid addition salt to the corresponding free base product.

13. A process according to any of claims 6 to 10 which includes the step of converting a product in the form of an acid addition salt to a non-toxic acid addition salt.

14. A pharmaceutical composition comprising a therapeutically effective amount of a compound as claimed in claim 1 and a pharmaceutical carrier or diluent.

15. A compound according to claim 1 as described in any of the foregoing Examples.

16. A process according to claim 6 substantially as herein described in any of the foregoing Examples.

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