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TECNICAL REPORT OF US AID

GRANT NO. DAN-5053-G-00-1071-00

OF PROJECT ENTITLED

ISOLATION AND SYNTHESIS OF POTENTIAL ANTIINFECTIVE AGENTS

SUBMITTED BY

DR. GOVIND J. KAPADIA

PRINCIPAL INVESTIGATOR

COLLEGE OF PHARMACY & PHARMACAL SCIENCES

HOWARD UNIVERSITY

WASHINGTON, D. C. 20059

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1E. **Technical Reporting**

1E. 2. (a) Quaterly Report

i) Characterization of the secondary reaction products isolated from the reaction mixtures of 4-allyl-1,2-dimethoxybenzene and safrole (3-allyl-1,2-methyenedioxy-benzene) under Friedel-Crafts reaction conditions has been completed and the results are presented in the attached Figures 1 & 2.

ii) The reaction conditions for Grignard reaction of 3′,4′-dimethoxy and 3′,4′-(methylenedioxy)-acetophenones (**1** & **2**) with 2-(2-magnesium bromide ethyl)-1,3-dioxolane (**3**) have been standarized (Figure 3). The major adducts (**4** & **5**) so formed were hydrolyzed, cyclized and aromatized into 1-methyl-6,7-dimethoxy and 1-methyl-6,7-(methylenedioxy)-naphthalenes (**6**) and (**7**) in quantitative yields. The desired products were characterized by spectroscopic analysis.

iii) Attempts to oxidize methyl group in naphthalenes (**6**) and (**7**) with a number of oxidizing agents (such as SeO2, SeO2 and t-BuOOH mixture, pyridium chlorochromate and DDQ) have been done. But none of the reagents has given satisfactory yields of the desired aldehyde. Improvization of oxidation conditions is still under progress to obtain the desired naphtthalene aldehyde.

iv) In view of the hindrance at oxidation stage, it has been thought to change the origional approach via two ways.

(a) Functionalization of the methyl group in compounds (**6**) and (**7**) by bromination followed by displacement with cyano function.

(b) Generation of naphthalene side chain via Friedel-Crafts reactions of 4-allyl-1,2-dimethoxybenzene and safrole with ethyl oxalyl chloride.

Preliminary reactions done by the change of strategies (a) and (b) appear to be promising. Conditions are being worked out for these alternative routes.

(v) Fractionation of Alchornia cordifolia

Alchornia cordifolia root powder (500 g) was extracted in a continuous extractor for two days with petroleum extract. This extract on concentration furnished 0.68 g of a precipitate (**Aco-1**) on filtration. The filtrate on evaporation and drying yielded 1.81 g residue (**Aco-5**). The plant material left after extraction with petroleum ether was extracted in continuous extractor with methanol. The methanol extract on concentration and drying furnished 63.11 g residue (**Aco-10**). This residue was shaken with 500 ml water acified to pH 3.5 with oxalic acid solution, centrifuged and supernatant separated by filtration. The insoluble residue was washed several times with water and dried to furnish 14.5 g of residue (**Aco-25**). The acidic filtrate and washings were combined and extracted in a liquid/liquid extractor with methylene chloride; the extract obtained on concentration and drying afforded

1.06 g of an alkaloidal fraction (**Aco-10A**). THe aqueous acidic layer remaining after extraction with methylene chloride was basified with ammonium hydroxide and extracted again with methylene chloride. The methylene chloride extract on concentration and drying provided a second alkaloidal fraction (**Aco-30**) weighing 0.090 g. The two alkaloidal fractions contained mixture of alkaloids and are currently being purified to obtain the pure compounds and evaluate their antimicrobial activity.

1E. 2. (b) Not applicable.

1E. 2. (c) Not applicable.

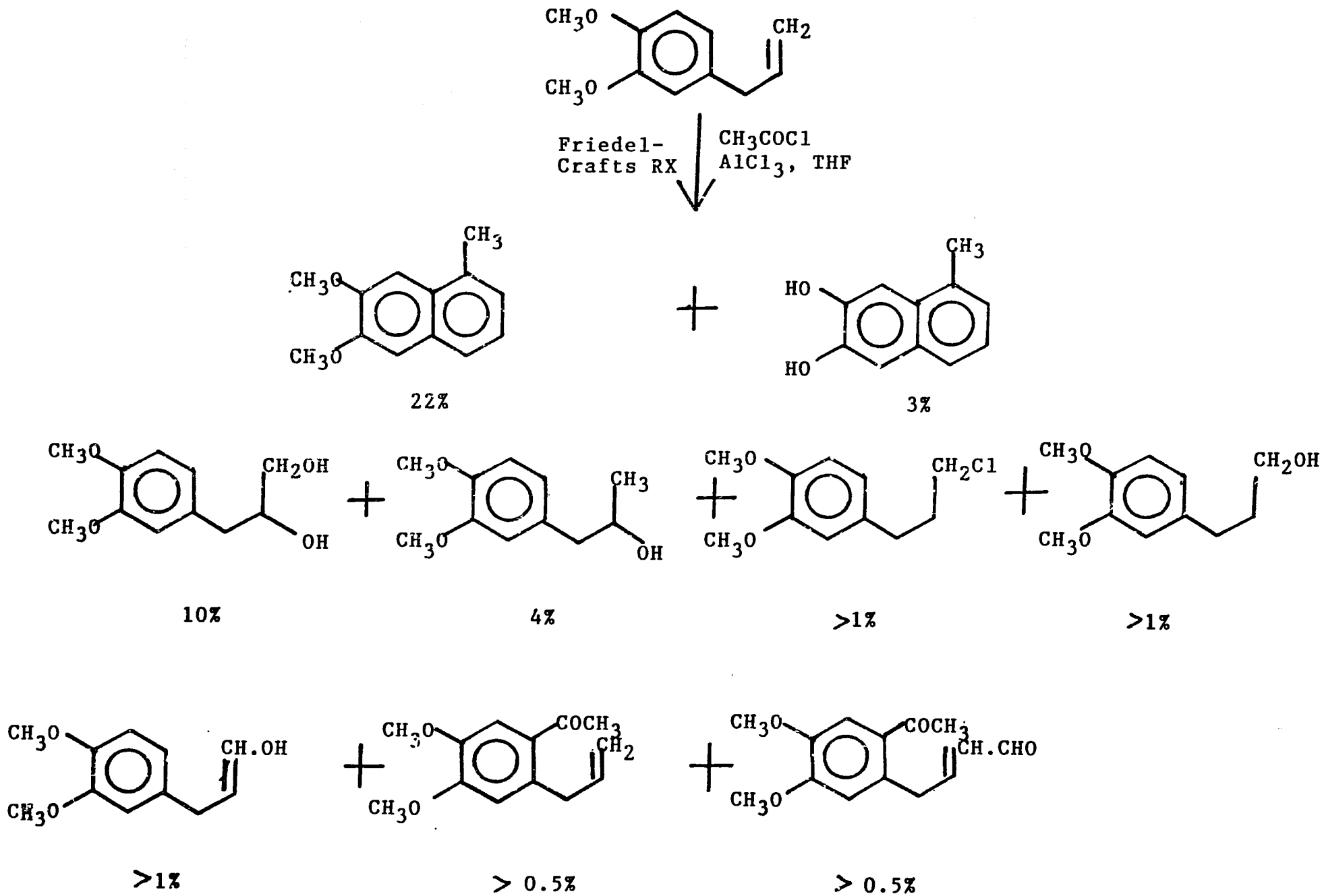
CH3O
CH3O
CH2
Friedel-
Crafts RX
CH3COCl
AlCl3, THF
CH3
CH3O
CH3O
22%
CH3
HO
HO
3%
CH3O
CH3O
CH2OH
OH
10%
CH3O
CH3O
CH3
OH
4%
CH3O
CH3O
CH2Cl
>1%
CH3O
CH3O
CH2OH
>1%
CH3O
CH3O
CH.OH
>1%
CH3O
CH3O
COCH3
CH2
> 0.5%
CH3O
CH3O
COCH3
CH.CHO
> 0.5%

Figure 1

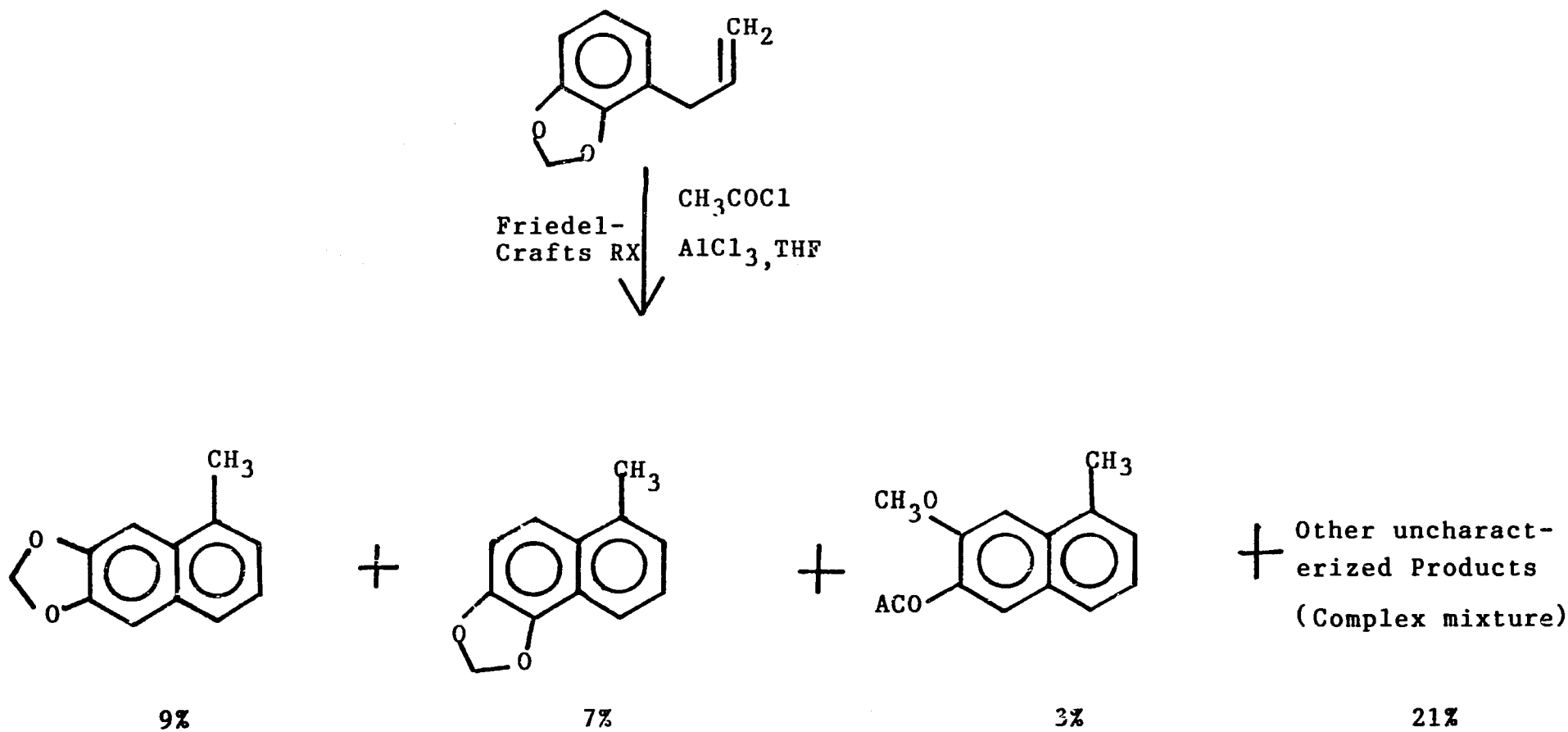
CH2
Friedel-
Crafts RX
CH3COCl
AlCl3,THF
CH3
CH3
CH3O
ACO
CH3
Other uncharact-
erized Products
(Complex mixture)
9%
7%
3%
21%

Figure 2

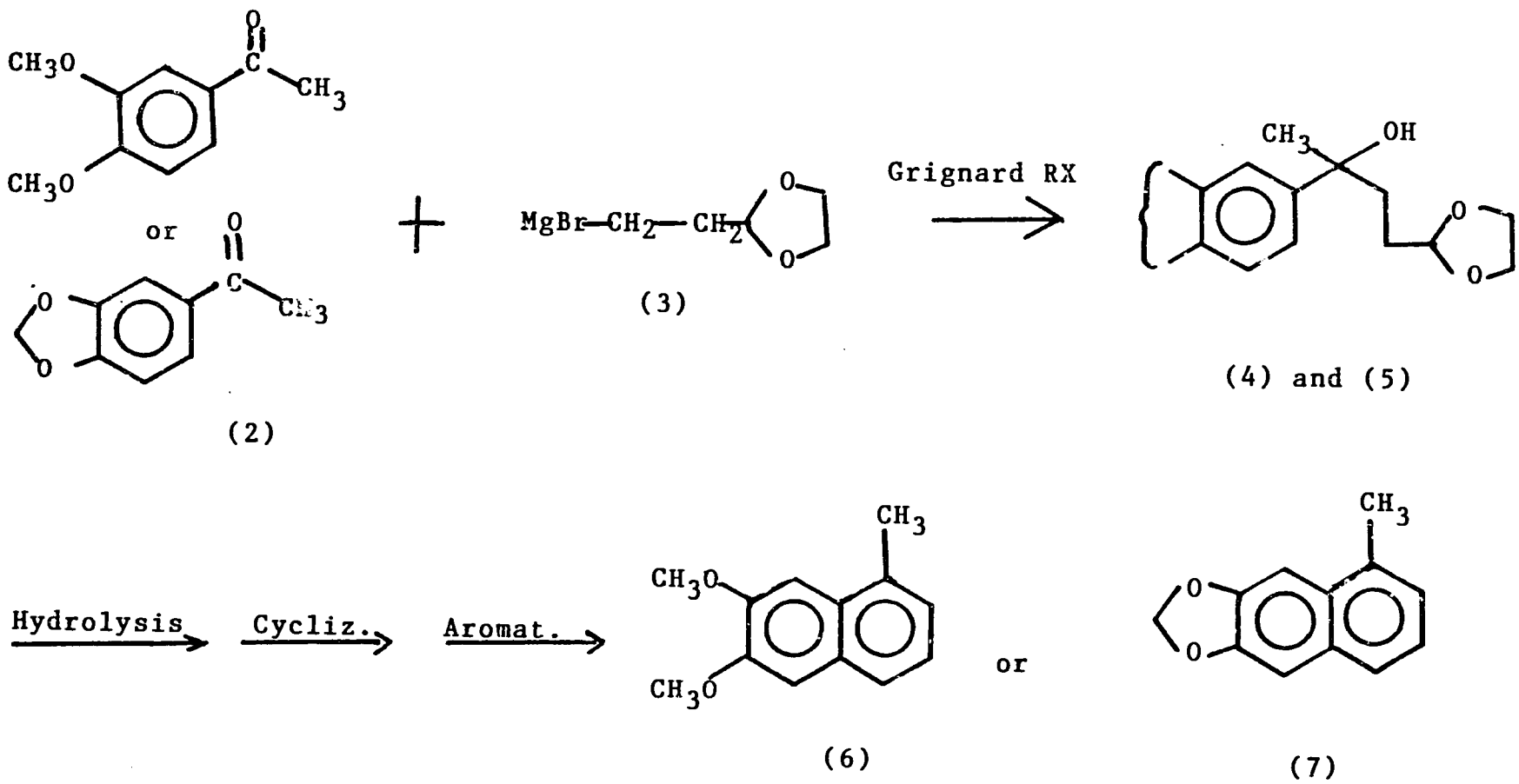
CH3O
CH3O
O
C
CH3
or
O
C
CH3
O
O
(2)
MgBr—CH2—CH2
O
O
(3)
Grignard RX
CH3
OH
O
O
(4) and (5)
Hydrolysis
Cycliz.
Aromat.
CH3
CH3O
CH3O
(6)
or
CH3
O
O
(7)

Figure 3

6

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DOXORUBICIN (DOX) UPTAKE BY P388 CELLS: INFLUENCE OF MODIFIED IONIC MILIEU AND MEMBRANE POTENTIAL.

L. Gailis and A. Thomas. Institut de Cardiologie de Quebec and Dept. of Medicine, Laval Univ., Quebec, Que, Canada G1V 4G5

In sarcoplasmic vesicles, doxorubicin uptake is highly dependent on imposed membrane potential. We wished to see whether a similar dependence on potential could be seen in intact cells. P388 cells were incubated 1 h in control Krebs-HEPES buffer [Na], or with NaCl replaced by KCl [K] (to decrease membrane potential) or Na_2SO_4 [SO_4] (to increase potential). Cellular DOX uptake was measured by separating cells by filtration. Binding to cellular DNA was measured by loss of DOX fluorescence. Results are expressed as Mean±SE, n=5-6. [K] did not significantly affect 5 uM DOX uptake by cells (109±11% of [Na]), or binding of 1 uM DOX to DNA (83±13%). However, [SO_4] increased binding to DNA (128±5%, $p<0.02$). Although possible confounding effects on cell viability need to be considered, the results indicate that the effect of membrane potential is not as large in intact cells as in sarcoplasmic vesicles. The results are not completely unexpected, given the much higher volume/surface ratio in cells.

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ANTIFUNGAL ACTIVITIES OF THE WATER EXTRACT OF MITRACARPUS SCABER.

C.O. Crockett, F. Guede-Guina, D.A. Pugh, M. Yangah-Manda, B. Bodo, M.O. Smith and R.F. Ochillo. *Laboratories of Pharmacology & Toxicology, Biomedical Research Center, Xavier University of Louisiana, New Orleans, LA 70125 U.S.A. and Laboratoire du Chimie du Museum D'Histoir Naturelle, 63, Rue Buffon, Paris 5.*

In certain parts of Africa, water extract of Mitracarpus scaber (Misca), a Rubiaceae, is used as folk medicine to treat skin diseases like tinea, scurf and candidiasis. Therefore, in our continuing search of antifungal agents, we used the agar slant method to investigate the antifungal activities of the water extract of Misca against Microsporum nanum (Mn), Trichophyton mentagrophytes (Tm) and Candida albicans (Cas). Traditionally, consideration of antifungal activities are often compared with antibacterial activities. Therefore, we also assessed the activities of the extract against Escherichia coli (Ec). The results are presented in the Table. The antifungal activities of the aqueous extracts of Misca are very potent against the investigated fungi suggesting the presence of active ingredient(s) with great therapeutic potential. The need for separation of the active ingredients is strongly indicated. The data provide an excellent scientific basis for the use of the extracts in folk medicine. (Supported by RCMI Grant #SRR 1G12 RR05750 from NIH and Prof. Guede-Guina was supported by Fulbright Grant #13420 from CIES).

INHIBITORY INDEX (mg/ml)	MICROORGANISM			
	Mr	Tm	Cas	Ec
MIC	< 0.098	< 0.049	0.09	0.195
MFC	0.195	0.098	25	17.5
IC_{50}	0.098	0.049	12.5	0.25

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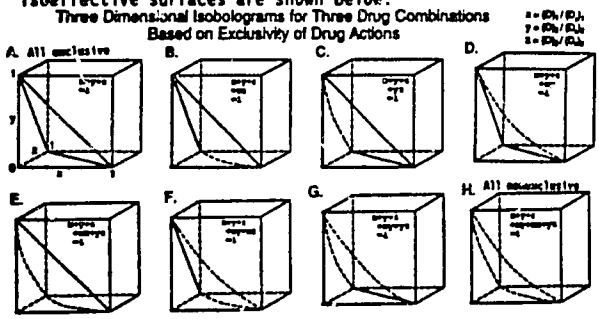
Theoretical basis and equations for three dimensional isobolograms for three drug combinations.

T.-C. Chou, and J.H. Chou. Mem. Sloan-Kettering Cancer Ctr, NY, NY 10021

The equations for generating two dimensional isobolograms based on receptor theory and enzyme kinetic models of the mass-action law had been derived (Chou & Talalay, 1981, 1984) and the computer software had been developed (Chou & Chou, 1986, 1991). This analysis extends and solves the dose-effective relationship for mutually exclusive and mutually non-exclusive drugs in three drug combinations. The combination index equation appears a simpler and more practical method to use. Eight patterns of isoeffective surfaces are shown below:

Three Dimensional Isobolograms for Three Drug Combinations Based on Exclusivity of Drug Actions



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PERTUSSIS TOXIN B CHAIN IS A POTENT BIOLOGICAL RESPONSE MODIFIER.

S. Graves, D. Stone, K. Kaushansky and A. C. Morgan Jr. NeoRx Corporation, 410 W. Harrison St. and University of Washington, Seattle WA 98119.

Bacterial and plant toxins are a rich source of biological response modifier (BRM) activity. In particular, Pertussis toxin affects a variety of immune responses including synthesis of IL-2, proliferation of lymphocytes and granulopoiesis. Here we demonstrate that the nonenzymatic B oligomer of Pertussis toxin (PtxB) has mitogenic activity and induces proliferation of both murine spleen cells and human peripheral blood lymphocytes. PtxB significantly enhanced cytotoxic activity (LAK) of 5 day cultures of nonadherent human peripheral blood lymphocytes and induced secretion of IL-1 from 20 h monocyte cultures. Northern blot analysis of RNA extracted from 20 h cultures of human monocytes revealed that PtxB (1 ug/ml) induced transcription of IL-1B but not IL-1α. Since IL-1 is known to affect hematopoiesis, we sought to utilize PtxB in ameliorating drug-induced myelosuppression in a murine model system. In these studies, a single iv injection of PtxB (5 ng) induced 7 day CFU-C from femoral bone marrow. Furthermore, injection of PtxB protected mice from cyclophosphamide-induced (200 mg/kg) myelosuppression. Administration of both PtxB and G-CSF to cyclophosphamide-suppressed mice induced granulopoiesis to a greater extent than G-CSF alone. Multiple injections of PtxB in cyclophosphamide-treated mice failed to induce anti-PtxB antibody. These data suggest that the B oligomer of Pertussis toxin may be a pharmaceutically acceptable BRM.

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EFFECTS OF ANTIULCER DRUGS ON ^{51}Cr ABSORPTION, RETENTION AND URINARY EXCRETION IN RATS.

M. T. Spicer, B. J. Stoecker, M. Orina, and S. Sanglah. Oklahoma State Univ., Stillwater, OK 74078

Thirty rats were fasted 12 h and intubated with sucralfate (30 mg/kg body wt), cimetidine (25 mg/kg body wt), omeprazole (2 mg/kg body wt), an over-the-counter antacid (OTCAA) (80 mg AlOH, 80 mg MgOH and 8 mg simethicone/kg body wt) or water followed by an oral dose of 100 uCi of $^{51}CrCl_3$. Blood samples were collected under light anesthesia by cardiac puncture at 0.5, 1, 2, 4, and 6 h post treatment. Animals were necropsied at 6 h. Urine, heart, liver, spleen, kidney, testes and bone were sampled to count ^{51}Cr. Blood ^{51}Cr was lowest ($p<0.05$) in rats dosed with OTCAA. Rats dosed with sucralfate (an aluminum salt of sucrose octosulfate) had higher ^{51}Cr in blood than the OTCAA group but lower than groups dosed with water (control), cimetidine (a histaminergic-H_2 type receptor antagonist), or omeprazole (a proton pump inhibitor). Rats dosed with OTCAA or sucralfate had lower ^{51}Cr in urine, liver and kidney than controls. The OTCAA group also had lower ^{51}Cr in bone and testes than controls. Cimetidine and omeprazole did not significantly alter ^{51}Cr in urine or tissues. The marked reduction in ^{51}Cr absorption when dosed with OTCAA or sucralfate suggests potential risk for impaired Cr status with use of these drugs (Supported by Oklahoma Agricultural Experiment Station, Hatch #2041).

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DESIGN OF NEW ANTIPROTOZOAL DRUGS BASED ON SELECTED NATURAL PRODUCT PROTOTYPES

K.K. Bhutani and G.J. Kapadia (SPON: S.M. Bhathena) Howard Uni. Coll. of Pharmacy Washington, D.C.

With the acceptance of artemisinin as a significant antimalarial drug, there has been resurgence of interest for natural products.

From the earlier work reported by Bhutani et al, SAR of phenanthroindolizidine alkaloids isolated from Tylophora species were deduced and common structural determinants of amoebicidal activity with emetine were found. Similarly, parthenin, an α-methylene-γ- lactone sesquiterpene isolated from Parthenium hysterophorus was shown to possess antiamoebic activity. The principle factors responsible for the activity have been recognised in this molecule as well from SAR. However, the therapeutic index in these prototypes was low.

To improvise this drawback, molecular modelling study has been done and computer graphics are drawn. This study has provided a new insight to the structural requirements (in the prototypes) for triggering the biological response. These requirements for the activity could be fulfilled by simplification of the structures of the prototypes. Theoretically, structural requirements match with other natural product prototypes namely anemone and artemisinine.