SHORT REPORTS

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MUTAGENIC AND ANTIBACTERIAL ACTIVITY TESTING OF NIMBOLIDE AND NIMBIC ACID

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Abstract

No mutagenicity of nimbolide and nimbic acid was detected by Ames'test using both TA98 and TA100 tester strains. However, nimbolide (0.875 mg/disk) did exhibit antibacterial activity (3/17 strains) against S. aureus, S. coagulase (+) *and* S. coagulase (-) *whereas nimbic acid (3.5 mg/disk) exhibited this activity (5/17 strains) against* S. aureus, B. subtilis, S. coagulase (+), S. coagulase (-) *and* Diphtheroidae.

Introduction

Sadao tree *(Azodirachta indica* var. *Siamensis)* is a tropical plant, the leaves of which are used as food and herbal medicine against malaria in Asia and Africa[1,2]. A crude ethanol extract of the leaves and the chemical nimbolide (Fig. 1), a major constituent, were found to exhibit antimalarial activity *in vitro* (Rochanakij, S. and Y. Yuthavong, personal communication). The latter inhibited growth of *Plasmodium berghei* at a concentration of 0.95 μ g/ml, but it was inactive against malaria when given per os (P.O.) (12.5 mg/kg/day) to the mice infected with *P. berghei* (Rochanakij, S. and Y. Yuthavong, personal communication). In contrast, nimbic acid (Fig. 1), a synthetic derivative of nimbolide, had antimalarial activity in the mice when given P.O. (Pinitglang, S and Y. Yuthavong, personal communication). Recently, the toxicity of these compounds was reported and it was found that nimbolide had LD_{50} values of greater than 600 and 225 mg/kg body weight (BW) when given intraperitoneally (I.P.) to rats and mice, respectively. Nimbolide was least toxic (LD_{50} > 600 mg/kg BW) when given P.O. to mice. Nimbic acid was even less toxic with LD_{50} values of greater than 600 mg/kg BW when given I.P. or P.O. to

mice[3]. With these reported biological activities, it was of interest to further evaluate the mutagenic and antibacterial activity of these compounds and of crude ethanol extracts to obtain possible supportive information towards the potential use of nimbolide and nimbic acid as antimalarial and antibacterial drugs.

Mutagenic activity was determined by the *Salmonella*/mammalian microsome mutagenicity test or Ames'test[4] using *S. typhimurium* of both TA98 and TA100 strains (kindly provided by Prof. B.N. Ames, University of California, Berkeley, U.S.A.) with or without S - 9 fraction from livers of male rats pretreated with polychlorinated biphenyl (PCB). Crude ethanol extracts from 1 - 10 mg/plate, nimbolide from 0.1 - 2.0 mg/plate and nimbic acid from 0.15 - 1.5 mg/plate were used. No mutagenic activity was exhibited by crude ethanol extracts, nimbolide or nimbic acid against both TA98 and TA100 tester strains either in the presence or absence of metabolic activation. Although crude ethanol extracts seemed to give a small increase in the number of his^+ revertants over the spontaneous rate in both TA98 and TA100, they were not denoted as mutagens since the increase was not dose - dependent and was not greater than 2 fold over the control values (Table 1). However, higher concentrations of both crude ethanol extracts and nimbolide were quite toxic to both TA98 and TA100 tester strains, particularly those incubated in the absence of metabolic activation.

Antibacterial activity was tested with 17 strains of bacteria (obtained from the Department of Microbiology, Faculty of Science and Department of Pathology, Faculty

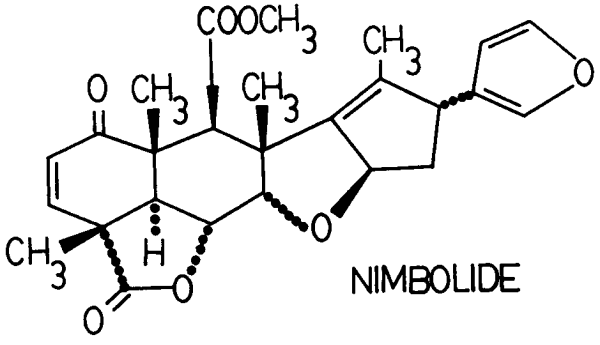

Fig. 1. Chemical structures of nimbolide and nimbic acid.

TABLE 1. MUTAGENICITY OF NIMBOLIDE, NIMBIC ACID AND A CRUDE ETHANOL EXTRACT FROM *A. INDICA* VAR. *SIAMENSIS* IN THE *SALMONELLA* / MAMMALIAN MICROSOME MUTAGENICITY TEST.

Chemical	Amount (mg/plate)	Number of His^+ revertants/plate* (TA98) -S-9 mix	(TA98) + S-9 mix	(TA100) -S-9 mix	(TA100) + S-9 mix
Crude extract	1.0	33	56	144	201
	2.5	28	68	153	184
	5.0	25	61	150	150
	10.0	0^K	52	133^{pK}	136^{pK}
Nimbolide	0.1	55	39	158	148
	0.4	52	41	157^{pK}	180
	1.0	55	35	153^{pK}	149
	2.0	40	37	53^K	80^K
Nimbic acid	0.15	36	39	145	149
	0.6	37	40	150	137
	1.5	31	37	154	145
Positive controls :					
AF - 2	0.02 (μg)	ND	ND	917	ND
AF - 2	0.20 (μg)	408	ND	ND	ND
Aflatoxin B_1	0.03 (μg)	ND	498	ND	1062
Benz (a) pyrene	1.0 (μg)	ND	150	ND	484
Solvent control :					
DMSO	100 (μl)	42	41	159	150

*Results are expressed as means of 2 experiments. S-9 mix contained 100 μl/plate of a PCB - induced rat liver S-9 fraction and an NADPH - generating system as used by Maron and Ames[4].

K = killing effect, pK = partial killing effect, ND = not determined, AF-2 = 2-2(furyi) -3-(5-nitro-2-furyl) acrylamide.

TABLE 2. ANTIBACTERIAL ACTIVITY OF NIMBOLIDE, NIMBIC ACID AND A CRUDE ETHANOL EXTRACT FROM *A. INDICA* VAR. *SIAMENSIS.*

Bacteria	Average inhibitory zone diameter (mm)[a]								
	CE	Te	E	NIM	Te	E	NA	Te	E
Bacillus subtitis	17.3	30.0	30.0	-	32.5	31.5	9.5	31.0	31.0
Diphtheroidae	16.0	17.5	17.0	-	19.0	18.0	11.0	17.0	18.0
Staphylococcus aureus	15.5	31.0	30.5	9.0	28.0	29.0	9.5	32.0	29.0
Staphylococcus coagulase (+)	17.3	11.5	29.5	9.5	13.0	27.0	10.5	13.5	32.5
Staphylococcus coagulase (-)	15.3	15.0	27.5	10.3	13.0	28.0	11.0	12.5	26.0

[a]CE, crude ethanol extract. NA, nimbic acid. NIM, nimbolide. E, erythromycin, Te, tetracycline. The amounts of chemicals were as follows : crude extract, 3.33 mg/disk; nimbolide, 0.875 mg/disk; nimbic acid, 3.5 mg/disk; erythromycin, 15 μ g/disk and tetracycline, 30 μ g/disk. Solvent control, DMSO 10 μ l/disk.

of Medicine, Ramathibodi Hospital, Mahidol University) by dropping the solutions of crude ethanol extracts (3.33 mg/disk), nimbolide (0.875 mg/disk) and nimbic acid (3.5 mg/disk) in DMSO (10 μ l) on paper disks. Two disks of each type were then transfered to the surface of seeded agar plates and clear inhibition zones were saught after suitable incubation. In each plate, one tetracycline disk (30 μ g) and one erythromycin disk (15 μg) were also plated as positive controls. No antibacterial activity was exhibited by all fractions tested against 12 bacteria including *Citrobacter, Escherichia coli* (2 strains), *Enterobacteri, Klebsiella pneumoniae, Proteus mirabilis, Proteus morganei, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas* E01, *Salmonella typhimurium* and *Streptococcus faecalis.* However, crude ethanol extracts and nimbic acid did exhibit a weak antibacterial activity against 5 bacteria including *Bacillus subtilis, Diphtheroidae, Staphylococcus coagulase (+), S. coagulase (-)* and *S. aureus,* while nimbolide exhibited activity against only the 3 latter strains of *Staphylococcus* (Table 2). Nimbolide seems to have a higher antibacterial activity than does nimbic acid since it gives about the same inhibition zone as the latter at one quarter of the concentration.

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บทคัดย่อ

ได้ศึกษาฤทธิ์ก่อกลายพันธุ์โดยใช้วิธีของ Ames และศึกษาฤทธิ์ต้านแบคทีเรียของ ,nimbolide และ กรด nimbic พบว่าทั้ง nimbolide และ กรด nimbic ไม่มีฤทธิ์ก่อกลายพันธุ์ต่อแบคทีเรีย *Salmonella typhimurium* สายพันธุ์ TA 98 และ TA 100 แต่อย่างไรก็ตามพบว่าทั้ง nimbolide และ กรด nimbic มีฤทธิ์ต้านแบคทีเรียโดยที่ nimbolide (0.875 มก/แผ่น) สามารถต้านแบคทีเรียได้ 3 สายพันธุ์ จากทั้งหมด 17 สายพันธุ์ คือ *S. aureus, S. coagulase(+)* และ *S. coagulase(-)* ส่วนกรด nimbic (3.5 มก/แผ่น) สามารถต้านแบคทีเรียได้ 5 สายพันธุ์ ซึ่งได้แก่ *S. aureus, B. subtilis, S. coagulase(+), S. coagulase(-)* และ *Diphtheroidae.*