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SPECIALIA

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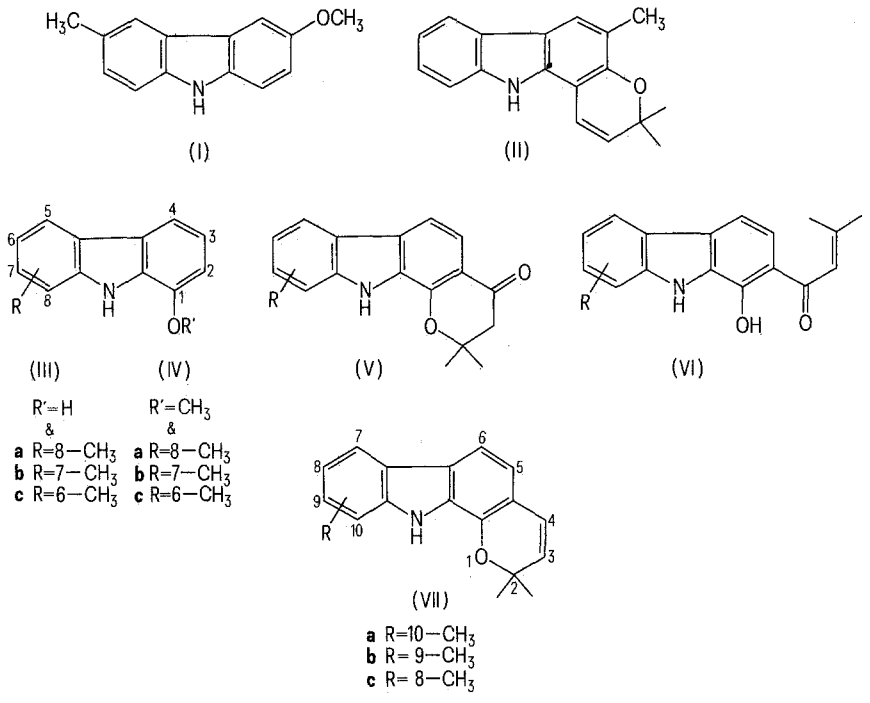
Antimicrobial activity of carbazole derivatives[1]

B.E. Randelia[2] and B.P.J. Patel[3]

Department of Chemistry, Indian Institute of Technology, Powai, Bombay-400 076 (India), 24 March 1981

Summary. Glycozoline and girinimbine isomers (**IV** and **VII**) were synthesized and their activity against 2 bacterial strains, viz., *E. coli* and *S. aureus,* and 2 fungal strains, viz. *C. albicans* and *A. niger* were studied. The hydroxy synthons (**III**) were also tested.

Carbazole derivatives exhibit diverse biological activities; for example they may be antidepressant[4], antiinflammatory[5], anticonvulsant[6], antiserotonin[7], cardiotonic[8,9], analgesic[9] etc. Some of their derivatives also display bactericidal[10], antiviral[11] and antifungal[12] properties. Chowdhury et al.[13], studied the insecticidal and antimicrobial properties of some carbazole, tetrahydrocarbazole and 1-oxo-tetrahydrocarbazole derivatives. The antibiotic activities of some carbazole alkaloids, e.g. glycozoline (**I**), and related compounds have been reported by Chakraborty et al.[14]. They found that demethylated glycozoline (6-hydroxy-3-methylcarbazole) was the most active antifungal agent among the



compounds tested, and that it compares well with griseofulvin, the antibiotic agent used in practice against the infection by *Microsporum, Epidermophyton* and *Trichophyton.* Das et al.[15], reported antimicrobial activities of some more carbazole alkaloids. Based on such reports and on the general features of carbazole alkaloids, we synthesized some carbazole and pyranocarbazole derivatives isomeric with the carbazole alkaloids glycozoline (**I**) and girinimbine (**II**). Their antibacterial and antifungal properties are recorded.

Materials and methods. The glycozoline isomers (**IV**) were synthesized in 5 convenient steps from cyclohexanone as reported earlier[16]. The girinimbine isomers (**VII**) were obtained in 3 steps from the respective synthons **III**. When 1-hydroxycarbazole derivatives (**III**) were reacted with β,β-dimethylacrylic acid under what has come to be called[17] the Grover-Shah-Shah conditions, the expected indolochromanone derivatives (**V**) were obtained along with the respective open chain isomers, 2-(3,3-dimethylacryloyl)-1-hydroxycarbazole derivatives (**VI**). Optimization of the ratio of the reagents, changes in the reaction conditions and modification of the work-up of the reaction afforded the indolochromanone derivatives (**V**) in about 55–70% yields. These were then reduced to the corresponding alcohols using sodium borohydride. The target compounds, girinimbine isomers (**VII**), were then obtained in 45–75% yields by in situ tosylation-elimination of the respective indolochromanol derivatives.

1-Hydroxycarbazole derivatives (**III**), glycozoline isomers (**IV**) and girinimbine isomers (**VII**) were tested against *Escherichia coli, Staphylococcus aureus, Candida albicans* and *Aspergillus niger.* The antimicrobial activity was evaluated[14] by the agar cup essay method utilizing nutrient agar for bacterial strains and Sabouraud's medium for fungal strains. The Petri dishes were incubated at 37 °C for about 16 h (except for *A. niger,* which was incubated for 2 days). The compounds were dissolved in a 1:4 mixture of ethanol and ethylene glycol. At least 3 trials were run for each concentration. Blanks for the solvent system were also run simultaneously.

Results and discussion. The results of the tests are presented in the table. It is observed that 1-hydroxycarbazole derivatives (**III**) are more active against the fungal strains than against the bacterial strains tested. Among these isomers, **IIIa** is found to be the most active. It appears that a symmetrical disposition of the methyl and the hydroxy groups on the carbazole ring is essential for maximum antifungal activity. Deoxygenated glycozoline also has a symmetrical disposition of these functionalities.

O-methylation **III** decreases the antifungal as well as antibacterial activity of the compounds (**IV**).

Concentrations: A = 2500 µg/ml, B = 1250 µg/ml, C = 625 µg/ml. Zone of inhibition given in mm (diameter)

Compound tested	*E. coli*			*S. aureus*			*C. albicans*			*A. niger*		
	A	B	C	A	B	C	A	B	C	A	B	C
IIIa	>20	20	18	>20	20	14	>20	>20	>20	>20	>20	>20
b	20	18	18	>20	18	16	>20	>20	20	>20	>20	>20
c	20	18	18	>20	20	18	>20	>20	20	>20	>20	18
IVa	14	10	--	18	12	--	12	10	--	10	--	--
b	16	10	--	18	12	--	--	--	10	--	--	--
c	14	14	10	--	--	--	10	--	--	10	--	--
VIIa	12	10	--	12	12	12	--	--	--	--	--	--
b	14	14	12	16	14	12	--	--	--	--	--	--
c	>20	20	16	>20	>20	>20	--	--	--	--	--	--

-- indicates < 10 mm zone.

The pyrano [2,3-a] carbazole derivatives (**VII**) were found to be active only against bacterial strains, more so against *S. aureus.* Among these isomers **VIIc** was found to be the most active.

Though the compounds **III a, b, c** and **IV a, b, c** showed antifungal and antibacterial activities respectively they do not compare very well with the standard antibiotics used in practice. However, from this lead further structural modifications should give promising antibiotics.

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2 Present address: Department of Life Sciences, Ramnarain Ruia College, Matunga, Bombay-400 019 (India).

3 Address for reprint requests: Research Centre, Hoechst Pharmaceuticals Ltd, L.B.S. Marg, Mulund, Bombay-400 080 (India).

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Glutathione and γ-glutamyl cycle enzymes in rat mammary gland*

J. Puente, E. Castellón and M. Sapag-Hagar

Departamento de Bioquímica, Facultad de Ciencias Básicas y Farmacéuticas, Universidad de Chile, Casilla 233, Santiago 1 (Chile), 31 August 1981

Summary. The main enzymes of the γ-glutamyl cycle during the lactogenic cycle in rat mammary gland were studied. A significant increase was found in all of them with the onset of lactogenesis. The effect of methionine sulfoximine on reduced glutathione concentration was studied in tissue slices of lactating mammary gland. The findings suggest that this compound affects glutathione synthesis by inhibiting γ-glutamylcysteine synthetase.

Reduced glutathione (GSH) is a tripeptide widely distributed in almost every living tissue at concentrations varying between 0.4 and 12.0 mM. Its intracellular level changes with growth, nutritional state and hormone levels in the organism[1,2]. In a tissue like mammary gland (in which its metabolism has been little studied), GSH concentration reaches 3.0 mM in the lactating gland[3].

Participation of GSH in different metabolic processes is determined by 2 of its most important structural characteristics, the presence of a γ-glutamyl bond and a SH group. The γ-glutamyl bond protects GSH against degradation by α-peptidases, and the only way to degrade it is by γ-glutamyltranspeptidase, the enzyme which initiates the γ-glutamyl cycle in a sequence of 6 reactions which allows GSH resynthesis and the transport of amino acids into the cell[4]. The presence of the SH group permits its participation in the protection of thiol groups in several detoxification reactions[5].

The γ-glutamyl cycle has been fully described in rat kidney, liver and brain and partially in erythrocytes and lens[6,7]; in general these tissues also have a high level of GSH. The cycle is regulated by GSH which in certain concentrations inhibits the enzyme γ-glutamylcysteine synthetase[8]. This enzyme is also inhibited by the convulsivant methionine sulfoximine[9].

We have already studied in our laboratory the hormonal dependence and some properties of γ-glutamyltranspeptidase from rat mammary gland[3,10]. Now we are studying the main enzymes of the γ-glutamyl cycle (γ-glutamyltranspeptidase, 5-oxoprolinase and γ-glutamylcysteine synthetase) in order to obtain information on its physiological role during the lactogenic cycle. We have also looked at the effect of methionine sulfoximine in GSH levels in tissue slices of mammary gland in lactogenesis.

Material and methods. Virgin and primiparous Sprague-Dawley rats (180–200 g) were taken at different stages of pregnancy and lactation. During lactation they were always kept with up to 8–10 pups.

Tissue slices were obtained from 12 to 15 days lactating mammary gland by using a Stadie-Riggs tissue slicer. Slices (100 mg) were incubated in Krebs-Ringer bicarbonate buffer pH 7.4, gassed with 95% O_2-5% CO_2 and containing 5 mM of each of the constitutive amino acids of glutathione (glycine, cysteine and glutamate) or the 3 amino acids plus 5 mM methionine sulfoximine. The slices were incubated with continuous shaking, up to 1 h. The γ-glutamyltranspeptidase activity was assayed using L-γ-glutamyl-p-nitroanilide as the donor substrate of the γ-glutamyl group and glycylglycine as the acceptor[11], 5-oxoprolinase was evaluated by quantification with glutamate dehydrogenase of the glutamate produced[12], and γ-glutamylcysteine synthetase was assayed by precipitation of the product L-[U-^{14}C] glutamylcysteine as cadmium mercaptide and subsequent counting by liquid scintillation[13]. The activities were expressed in units/g tissue, 1 unit being defined as the amount of enzyme that catalyzes the formation of 1 μmole product/min/g tissue.

GSH was determined by the method of Ball[16]. L-[U-^{14}C]glutamate was obtained from the Radiochemical Centre, Amersham (285 mCi/mmole). L-γ-glutamyl-p-nitroa-