Table II. Incorporation of UDP-^{14}C-glucose into alkali-insoluble glucan by cell free extracts of *P. palmivora* zoospores

Cell fractions	Con A	Con A + Methyl-α-D-mannoside	Methyl-α-D-mannoside	No addition
1000 × *g* Pellet	3.38	4.09	4.0	3.41
10,000 × *g* Pellet	5.11	6.69	7.57	7.49
100,000 × *g* Pellet	4.62	5.35	5.38	4.33

The values are percent of radioactivity incorporated into alkali-insoluble glucan. The reaction mixture (final volume 0.6 ml) consisted of 0.29 μmoles of cellobiose, 3.4 μmoles of UDP-^{14}C-glucose (250,000 dpm), 10.8 μmoles $MgCl_2$, 0.083 *M tris*-HCl buffer, pH 7.5, and 0.1 ml particulate enzyme. Con A (200 μg in 0.41 *M* NaCl) and/or 0.16 *M* methyl-α-D-mannoside was added to the appropriate samples. The cell fractions were prepared as described elsewhere[6].

presence of Con A concentrations that caused total lysis of unencysted cells. It seems unlikely that the cyst wall protected the cell simply by blocking the entry of Con A since it can penetrate through the wall and bind onto the cyst plasmalemma[3].

Since Con A binds intensely to the amorphous material secreted by encysting zoospores, the possibility was considered that Con A provoked zoospore lysis by interfering with the process of cyst wall formation. However, Con A did not markedly inhibit the activity of wall-glucan synthetases in a cell free system (Table II). In this cell free system, glucose residues are joined by β, 1–3 and β, 1–6 but not by β-1, 4 linkages[6,7], hence, the possibility that Con A might block specifically, cellulose synthesis could not be excluded. Alternately, the primary effect may be on the zoospore plasmalemma where Con A may disrupt its function. For instance, it may adversely affect the discharge of peripheral vesicles and thus selectively upset the process of cell wall neogenesis of the zoospore without affecting wall formation in subsequent developmental stages.

Con A can sometimes be toxic[8] but, to our knowledge, the drastic lethal action recorded herein has not been formerly described. The lysis of zoospores by Con A invites questions on the occurrence of this phenomenon particularly during host/parasite interactions, and raises the speculation, that it might be another way in which lectins contribute to defend higher plants against pathogens[9].

Resumen. La concanavalina A causa la lísis total de las zoosporas de *Phytophthora palmivora*. Una vez enquistadas, las células se vuelven resistentes a esta lectina. Aparentemente, la concanavalina A interfiere especificamente con el proceso de neogénesis de pared celular.

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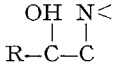
The Proposal of an Unified Model for the Interpretation of the Activity of Different Classes of β-Adrenergic Agents

The β-adrenergic agonists and antagonists can be divided[1] in two types, **A** and **B**. Although the structure-activity relationship of these drugs has been extensively studied, there is no satisfactory explanation for the way in which the Ar–O–CH_2 moiety of type **B** compounds can replace the single aromatic nucleus of type **A** agents in the drug-receptor interaction[2]. Therefore, we have initiated



A: R = Aryl; **B**: R = Aryl–O–CH_2

an investigation of the X-ray crystal structures of representative compounds of type **B**, and compared these three-dimensional structural data with similar kinds of data from type **A** compounds. The crystal structures of two type **B** β-blockers (propranolol [**2**] and alprenolol [**3**]) have been reported[3], and we have recently determined the structures of propranolol, propranolol hydrochloride and dichloroisoproterenol, a type **A** β-blocker.

The torsion angles about the C_1–C_2 bond are similar in both **2** and **2**·HCl, and correspond to a conformation in which nitrogen is approximately *anti*-periplanar to the

[1] For type **B** β-adrenergic agonits, see: J. A. EDWARDS, B. BERKOZ, G. S. LEWIS, O. HALPERN, J. H. FRIED, A. M. STROSBERG, L. M. MILLER, S. URICH, F. LIU and A. P. ROSZKOWSKI, J. med. Chem. *17*, 200 (1974).
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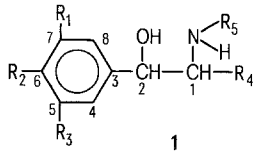
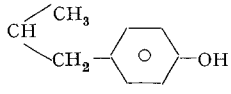
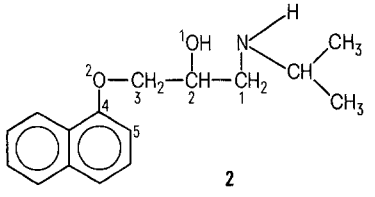
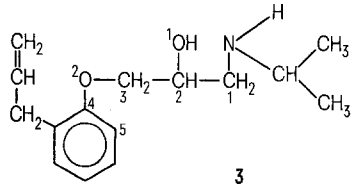
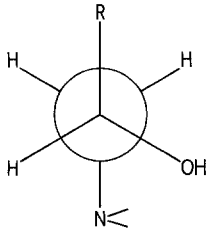
Dihedral angles between the least-squares planes of N–C_1–C_2–C_3 and C_3–C_4–C_5–C_6–C_7–C_8 for adrenergic drugs of the general formula **1** and of N–C_1–C_2–C_3 and C_3–O_2–C_4–C_5 for propanolol (**2**) and alprenolol (**3**)

Compound						Pharmacological action	Salt	Dihedral angle
	 **1**							
	R_1	R_2	R_3	R_4	R_5			
Norephinephrine	OH	OH	H	H	H	α-Agonist	Hydrochloride	82.6[5]
Epinephrine	OH	OH	H	H	CH_3	α,β-Agonist	Hydrogen (+)-tartrate	2.8[11]
Isoproterenol	OH	OH	H	H	$CH(CH_3)_2$	β-Agonist	Sulfate dihydrate	78.4, 74.7[6]
Dichloroisoproterenol	Cl	Cl	H	H	$CH(CH_3)_2$	β-Antagonist	Free base	84.1[14]
Ephedrine	H	H	H	CH_3	CH_3	α,β-Agonist	Hydrochloride	84.3[4]
							Monohydrogen phosphate monohydrate	87.0, 86.9[10]
Th 1165a	OH	H	OH	H		β-Agonist	Hydrobromide	72.9[7,9]
Salbutamol	CH_2OH	OH	H	H	$C(CH_3)_3$	β-Agonist	Free base	73.7[8,9]
 **2**					 **3**			
Propranolol (**2**)						β-Antagonist	Hydrochloride	56.6
							Free base	70.0
Alprenolol (**3**)						β-Antagonist	Hydrochloride	77.2[3b]

Ar–O–CH_2 moiety (structure **4**). This arrangement has been found in the solid state by X-ray crystallographic analyses[3–11], in solution by NMR-spectroscopy[12] and in the gas-phase by MO calculations[13] for α- and β-adrenergic agonists and for β-adrenergic antagonists. The conformations of the C_2–C_3, C_3–O_2 and O_2–C_4 bonds from the naphthyl–O–CH_2–CH(OH) moieties of type **B** drugs are of particular interest when compared to conformations in the aryl–CH(OH) portions of type **A** compounds, because it appears that the C_3–O_2–C_4–C_5 regions in the type **B** drugs are structurally analogous to the aryl nuclei of the type **A** drugs.



4, R = Ar–O–CH_2, Ar

In both the type **A** β-blocking drugs and α,β-stimulating agents (**1**, Table) the aromatic ring together with the side chain hydroxyl and nitrogen have been generally regarded as the main reactive centers for the interaction of the drug with the receptor. In order to explain the β-adrenergic blocking activity of the type **B** agents, it was suggested that the O–CH_2 bridge holds the aromatic group of these drugs in a position, relative to the aminoethanol side chain, which is similar to that of the aromatic ring directly linked to the side chain of the type **A** drugs[2d]. Other workers have observed that the insertion of the O–CH_2 group modifies the distances between the aromatic group and the other active centers of the drug, and have proposed, therefore, that complementary neighboring accessory receptor areas may be involved in the drug-receptor interaction[2b,3a].

Based on our results, we propose an alternative, and in our opinion more likely, hypothesis: that the O_2–C_4–C_5

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moiety in the type **B** β-adrenergic drugs, owing to the conjugation of oxygen with the naphthyl ring, can electronically and sterically simulate a portion of an aromatic ring and therefore take the place of the aryl group directly linked to the C_2 atom in the type **A** drugs in the interaction with the receptor. This hypothesis is supported by the fact that the relative orientations of the least-square planes of the C_3–O_2–C_4–C_5 group and the aminoethanol side chains are very similar in **2**, **2**·HCl and **3**·HCl, and that this same kind of orientation has been found between the aryl and aminoethanol groups in the majority of type **A** (**1**) drugs which have been examined by X-ray crystallography. The least-squares plane angle data reported in the Table show that there is a clear relationship between the Ar–CH(OH) and Ar–O–CH_2CH(OH) moieties: the angles are all in the 56–87° range, with the one exception of adrenaline tartrate (2.8°). Small differences observed in the angles are probably due to crystal packing forces[15].

Zusammenfassung. Mittels Röntgenstrahlen wurde die Kristallstruktur des β-Blockers Propranolol und seines Chlorhydrats vermessen und beim letzteren gewisse Unterschiede zu früheren Resultaten gefunden. Auf Grund dieser Vermessungen wird erklärt, weshalb auch der Typus B Antagonisten liefern kann, und eine neue Hypothese für die Wirksamkeit von Typ B aufgestellt.

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[15] We thank the University of Maryland Computer Science Center for a grant of computer time.

[16] [a] University of Maryland; [b] Università di Pisa; [c] Medical College of Virginia.

Properties of Ca^{2+}, Mg^{2+}-Dependent Endonuclease from Sea Urchin Eggs of *Arbacia punctulata*

A Mg^{2+}-dependent endonuclease is present in embryos of sea urchin (*Paracentrotus lividus*)[1], in testis of the crabs (*Neptunas astalus*)[2] and *Cancer pagurus*[3] and in the hepatopancreas of *Octopus vulgaris*[4] which requires Ca^{2+} for maximal activity. In the present study a deoxyribonuclease was demonstrated in eggs of the sea urchin *Arbacia punctulata* which required both Ca^{2+} and Mg^{2+} for activity. It was capable of stimulating the template activity of sea urchin sperm chromatin for DNA synthesis.

Materials and methods. Eggs and sperms were obtained from sea urchin by an established method of injecting 0.5 ml of 0.55 *M* KCl. The semen was suspended in a small amount of artificial sea water and centrifuged at a low speed. The supernatant was designated as seminal plasma. Sperm chromatin was prepared as described by OZAKI[5] with slight modifications.

Endonuclease was isolated from eggs which were washed several times with artificial sea water (MBL) and suspended in a cold medium containing 25 m*M* *Tris*-HCl (pH 8.0), 2 m*M* $MgCl_2$. The eggs were homogenized in a Dounce homogenizer. The homogenate was centrifuged at 15,000 *g* for 15 min. The supernatant was fractionated by precipitation with solid ammonium sulfate (50–80% saturation). The precipitated proteins were collected by centrifugation at 15,000 *g* for 15 min and dissolved in a medium containing 0.01 *M* *Tris*-HCl (pH 8.0), 30% glycerol.

Egg nuclei were prepared according to PIKO, TYLER and VINOGRAD[6]. The endonuclease was extracted from the isolated nuclei as described for rat testis nuclei[7]. The procedure for the determination of acid and alkaline endonuclease activities by measuring the amount of radioactivity solubilized from [^{3}H]DNA gel and the method for measuring the template activity of sperm chromatin with DNA polymerase and [^{3}H]TTP were described in previous reports[8–10].

DNA was prepared from sea urchin sperm chromatin as described by SMITH[11]. The endonucleolytic property of the egg enzyme was determined by incubating a mixture containing 100 μg of sea urchin sperm DNA, 10 m*M*

Table I. Effect of bivalent cations and EGTA on endonuclease activity in sea urchin eggs

Assay systems	Activity (10^{-3} units/mg protein)
Control	0
+ 10 m*M* $MgCl_2$	0.06
+ 2 m*M* $CaCl_2$	0.18
+ 10 m*M* $MgCl_2$ 0.6 m*M* EGTA	0
+ 10 m*M* $MgCl_2$ 2 m*M* $CaCl_2$	8.88
+ 10 m*M* $MgCl_2$ 2 m*M* $CaCl_2$ 0.6 m*M* EGTA	8.22

Control assay system contained 100 μl [^{3}H] DNA gel (2×10^4 cts/min) 50 m*M* *Tris*-HCl (pH 7.5), 10 m*M* 2-mercaptoethanol, appropriate amount of enzyme preparations and with or without 10 m*M* $MgCl_2$, and 0.6 m*M* EGTA in a total vol of 0.2 ml. The mixture was incubated at 37 °C.

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