

lost. The sediment which comprised of the head and midpiece fractions was washed twice with distilled water and further subjected to ultrasonication for a period of 15 seconds.

The ultrasonicated sample was layered on (1) M/15 phosphate buffer, pH 7.0, (2) M/50 phosphate buffer, pH 7.0, (3) seminal plasma of the same sample.

The samples were centrifuged at $250 \times g$ for 3 minutes. In the case of M/15 and M/50 phosphate buffer a broad layer was found in the medium and sizable amounts settled in the form of pellet. The layer was carefully removed with a pasteur pipette. Microscopical examination revealed that this layer comprised of midpieces while the pellet consisted of heads. There was considerable shrinkage in the structure of the midpiece and the heads. The possibility of shrinkage of the heads and midpieces due to the release of intracellular constituents as a result of hyperosmolarity is ruled out since M/15 or M/50 phosphate buffer was able to maintain the normal morphology and viability of the neat semen. The cross contamination between the head and midpiece was about 10%.

In the experiments with seminal plasma, all experimental conditions remained the same as described for the phosphate buffers with the exception that the suspending material was seminal plasma of the same sample. A distinct and sharp layer was formed which comprised of midpieces and a sizable amount settled down at the bottom consisting of heads. The normal morphology of the midpieces and heads was maintained and the cross contamination was approximately 5%. In subsequent experiments it was observed that the seminal plasma of other buffalo bulls or even bulls could also be used as suspending medium without altering the morphology of the heads and midpieces.

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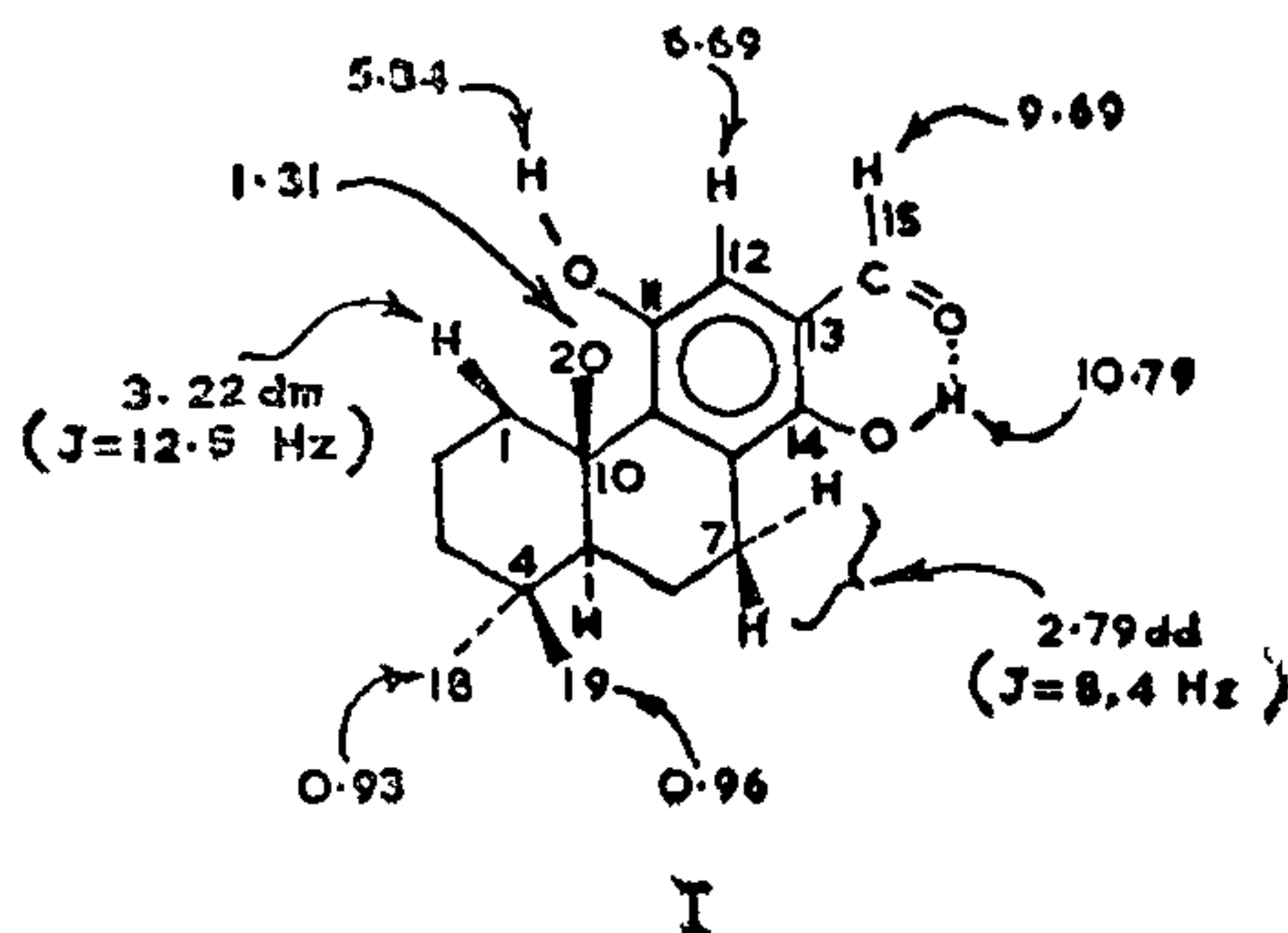
PREMNOLAL, A NEW AROMATIC BISNORDITERPENE FROM *PREMNA* *LATIFOLIA* ROXB.

THE *n*-hexane extract of dry root bark of *Premna latifolia* Roxb. (Verbenaceae) gave on chromatography over silica gel a light lemon yellow compound (I),

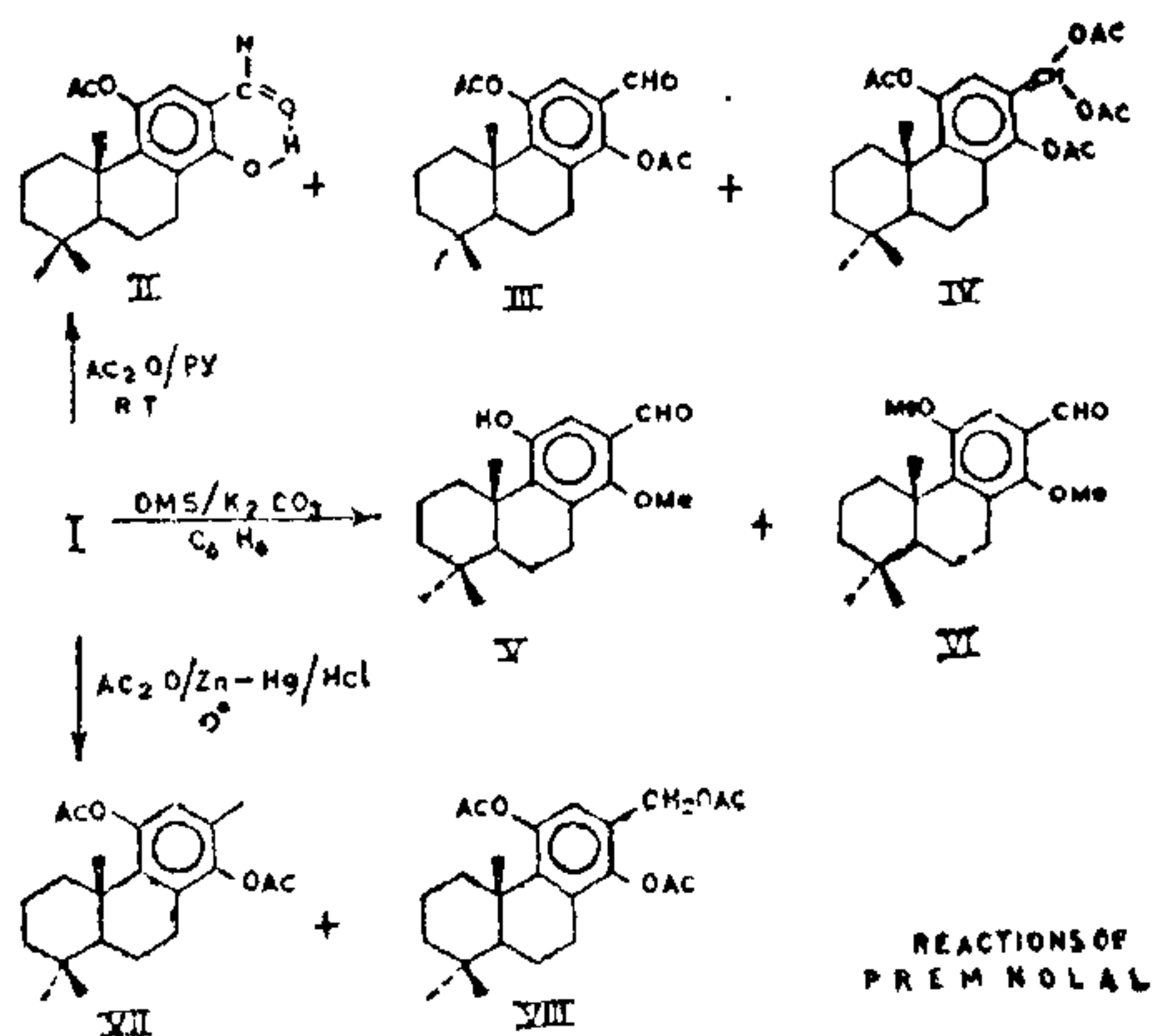
m.p. 135–135.5°; molecular formula, $C_{18}H_{24}O_3$ (*m/e* M^+ , 288); $[\alpha]_D^{20} + 67.5^\circ$ (*c*, 1.0, $CHCl_3$). It was found to dissolve slowly in aqueous alkali giving an orange yellow solution and gave a green ferric colour in alcohol which turns yellow quickly, indicating the presence of *p*-hydroxy phenolic function. On acetylation, it formed a mixture of three acetates, separated by chromatography over silica gel, (a) monoacetate (II), m.p. 144–145° ($C_{22}H_{28}O_4$); (b) diacetate (III), m.p. 147–148° ($C_{22}H_{28}O_5$) and (c) tetraacetate (IV), m.p. 137° ($C_{26}H_{34}O_8$). Methylation using dimethyl sulphate in presence of anhydrous potassium carbonate in boiling benzene yielded a mixture of monomethyl ether (V), m.p. 145° ($C_{19}H_{26}O_3$) and dimethyl ether (VI), liq. ($C_{20}H_{28}O_3$). It also formed a 2, 4-dinitrophenylhydrazone, m.p. 254° ($C_{24}H_{28}O_6N_2$) indicating the presence of carbonyl function. The compound is named 'premnolal' and its spectral characteristics are: U.V. λ_{max} nm (log ϵ) in cyclohexane or ethanol, 208 (3.69), 236 (4.14), 288 (4.16); in ethanol + one d.op of 10% aq. NaOH, 220 (4.74), 252 (4.30), 305 (3.99) and no change with trace of HCl; I.R. $\nu_{max}^{CCl_4}$ cm^{-1} 3550 (–OH), 3180–3120 (–OH, chelated), 1665, 1650 sh (*o*-hydroxyaryl aldehyde), 1385, 1360 (gem dimethyl) and in KBr, 1560 (aromatic) and 1H NMR (XL-100 spectrum in CCl_4 , δ) 0.93, 0.96, 1.31 (singlets, each 3H, $3 \times ter. CH_3$), 2.79 (*dd*, $J = 8, 4$ Hz, $Ar-CH_2$), 3.22 (*dm*, $J = 12.5$ Hz, 1H), 5.84 (*s*, –OH, disappeared with D_2O), 6.69 (*s*, $Ar-H$), 9.69 (*s*, –CHO) and 10.79 (*s*, chelated–OH, disappeared with D_2O), in addition there were signals which appeared in the methylene region between 1–2 δ integrating to eight protons.

From the data presented above, the constitution of premnolal has been deduced as tricyclic diterpene with aromatic ring–C and C-16 and C-17 missing, while C-15 is present as formyl group. The *p*-hydroxy phenolic function has been indicated by transient green ferric colour, besides negative Liebermann and Gibbs¹ tests for a free *p*-position to hydroxyl. The U.V. absorption bands at 288 nm in neutral and 305 nm in alkaline solutions are well within the limits of values of substituted benzaldehydes calculated² on the basis of two ring residues in *m* and *p*-positions and two hydroxyls in *o* and *m*-positions and this also precludes a *p*-hydroxyaldehyde system which is expected to produce a larger red shift on passing from neutral to alkaline medium. Further, hydroxyl function at 11 position is indicated by the appearance of a single proton signal at 3.22 δ assignable to $1\beta-H^?$. This leaves 12 and 13 positions for the formyl group, and the latter is preferred assuming an unrearranged diterpene system, which is further substantiated by the study of I.R. and 1H NMR spectra of the acetyl derivatives. The stereochemistry of premnolal is derived by the comparison of its dextro rotation with

derivatives of abeitatriene of $5\alpha:10\beta$ normal configuration at the AB ring junction⁴⁻⁶. Thus premnolal can be represented as 13-formyl-11, 14-dihydroxy-podocarpa-8, 11, 13-triene (I).



Premnolal on acetylation using acetic anhydride saturated with dry hydrogen chloride in presence of zinc amalgam at 0° gave two acetates: premnol diacetate (VII), m.p. $132-132.5^\circ$ ($C_{22}H_{30}O_4$) and 15-acetoxypremnol diacetate (VIII), liq. ($C_{24}H_{32}O_6$). VII was also prepared from premnolal by the sequence of reactions: reduction with sodium borohydride, tosylation, reduction using LAH followed by acetylation, and VIII by reduction with borohydride and acetylation. The structures of all the derivatives of I, assigned, are supported by their spectral data and analysis. The mass fragmentation pattern of premnolal (I) as well as premnol diacetate (VII) agreed well with the results for aromatic diterpenes of podocarpa-8, 11, 13-triene system published by Enzell *et al.*¹ and consistent with the structures assigned. The reactions of premnolal are summarised in the following chart.



Premnolal provides the first example of naturally occurring aromatic tricyclic diterpenes in which the

isopropyl group is degraded to formyl in 13 position which might involve dealkylation and formylation or direct oxidative mechanism. Fuller details will be published elsewhere.

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LIPOGENIC ACTION OF CYCLIC AMP IN VITRO

BLECHER¹ observed a stimulatory action of high concentrations (10 mM) of dibutyril adenosine 3', 5' cyclic monophosphate on lipogenesis from glucose in fact cell suspensions *in vitro*. In contrast, an *in vitro* lipolytic action of exogenous adenosine 3', 5' cyclic monophosphate (cyclic AMP) had also been demonstrated². I had noted lipogenic effect of cyclic AMP on the rat epididymal adipose tissue (EAT) segments *in vitro*. The results, along with the possibility of glycolytic kinases being a locus of lipogenic action of cyclic AMP *in vitro* are presented.

Results and Discussion

Details of experimental conditions for lipogenic studies and enzyme assays are described already³. In presence of 1 mM cyclic AMP, adipose tissue segments exhibited statistically significant higher (75% and 62%) lipogenic capacity (Fig. 1). 5'-adenosine monophosphate (5' AMP) lacked such a stimulatory