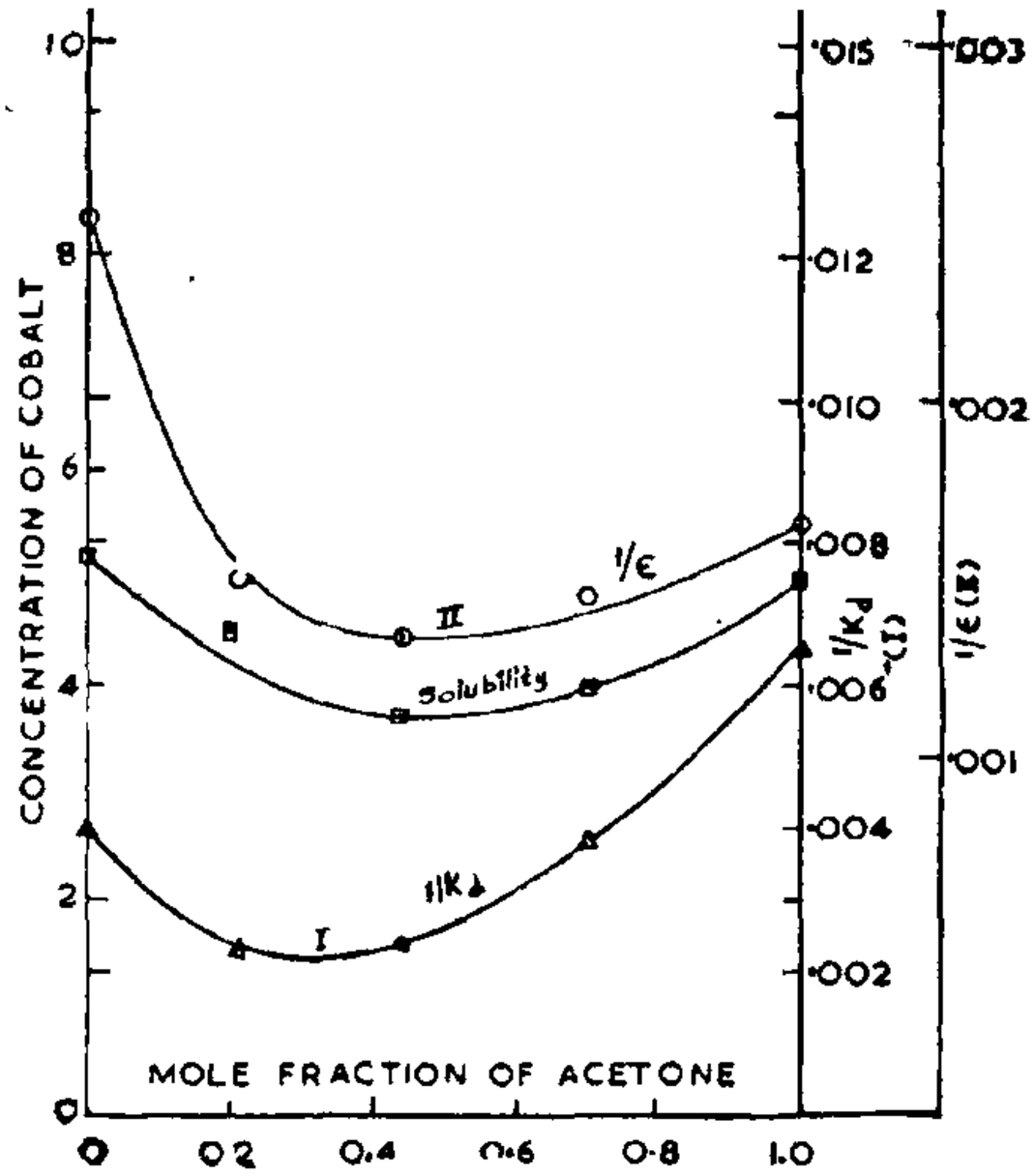

FIG. 1. Comparison of anion exchange behaviour of Co(II) with the solubility and spectral data of the cobalt (II) complex.

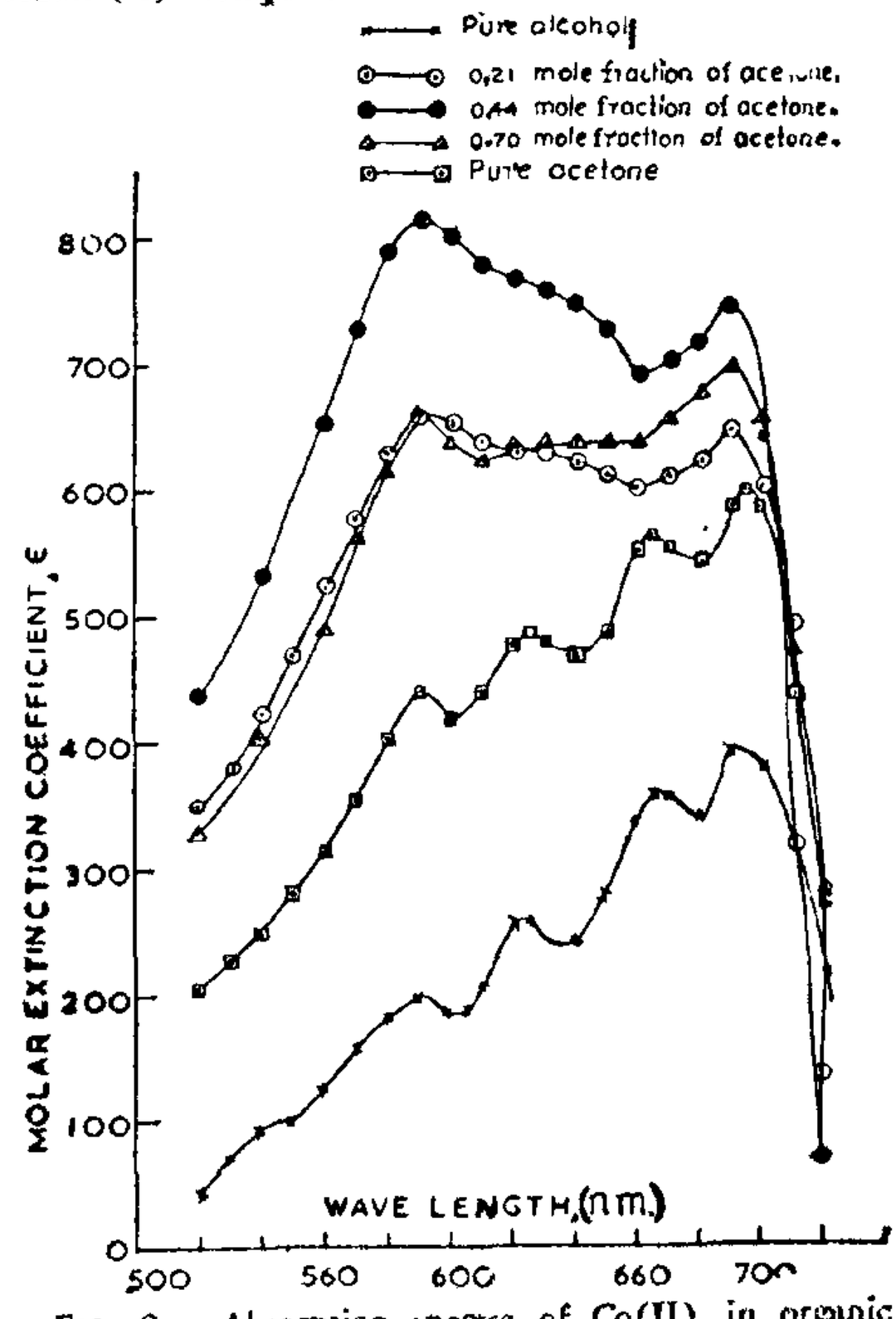

FIG. 2. Absorption spectra of Co(II) in organic solvent media.

greater in the mixed solvent media than in the individual solvents. The peak at 690 nm has been assigned to $CoCl_4^=$ species by Fine[4]. A plot of $1/\epsilon$ value at this wave length as a function of solvent composition (Fig. 1) also shows a trend similar to the solubility plot. It is thus possible to conclude the synergitic effect is due to the enhanced formation of $CoCl_4^=$ in mixed solvent media as a result of structural changes of the medium arising from solvent-solvent interactions.

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A METHOD FOR FRACTIONATION OF BUFFALO SPERMATOZOA SUITABLE FOR THE STUDY OF FRUCTOSE METABOLISM

Two different methods for fractionation of buffalo spermatozoa into head, midpiece and tail have been described ealier which essentially require different molar concentrations of sucrose either separately or in the form of a gradient as the separation medium[1,2] and are satisfactory for the assay of various enzymes in the different fractions[3]. However, both the techniques of fractionation were found to be unsuitable for the estimation of fructolysis in spermatozoal fractions and also such enzymes where the end product of the assay was fructose or glucose. Even repeated washings of the heads and midpiece fraction with distilled water or phosphate buffer did not eliminate the trace of sucrose present in the fraction.

Approximately 2 ml of freshly collected buffalo semen was centrifuged at 1400 × g for 10 minutes. The seminal plasma was decanted. The sperm pack was resuspended in an equal volume of distilled water and centrifuged again for 10 minutes at 1400 × g. This procedure was repeated twice. The washed sperm pack was diluted 3 fold with distilled water and disintegrated with the aid of vibronics ultrasonicator (model VPL Pi) for 60 seconds at a frequency of 25 kilocycles per second. The ultrasonicated sample was centrifuged at 2000 × g for 10 minutes in a refrigerated centrifuge. The supernatant was carefully drawn with the aid of a pasteur pipette. The supernatant comprised of tails which were fragmented almost to colloidal dimensions and its morphological entity was

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 × 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 hyperosmotic ity 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); $[\alpha]_D^{10}$ + 67·5° (*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° ($C_{22}H_{26}O_4$); (*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° ($C_{24}H_{28}O_6N_4$) 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 drop of 10% aq. NaOH. 220 (4·74), 252 (4·30), 305 (3·99) and no change with trace of HCl; I.R. ν_{max}^{CCl} 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 × *ter*. CH_3), 2·79 (*dd*, J = 8, 4 Hz, Ar–CH_2), 3·22 (*dm*, J = 12·5 Hz, 1 H), 5·84 (*s*, –O*H*, disappeared with D_2O), 6·69 (*s*, Ar–*H*), 9·69 (*s*, -C*H*O) and 10·79 (*s*, chelated–O*H*, 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 Leibermann and Gibbs[1] 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 calculated[2] 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 singal at 3·22 δ assignable to 1β–H^3. 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