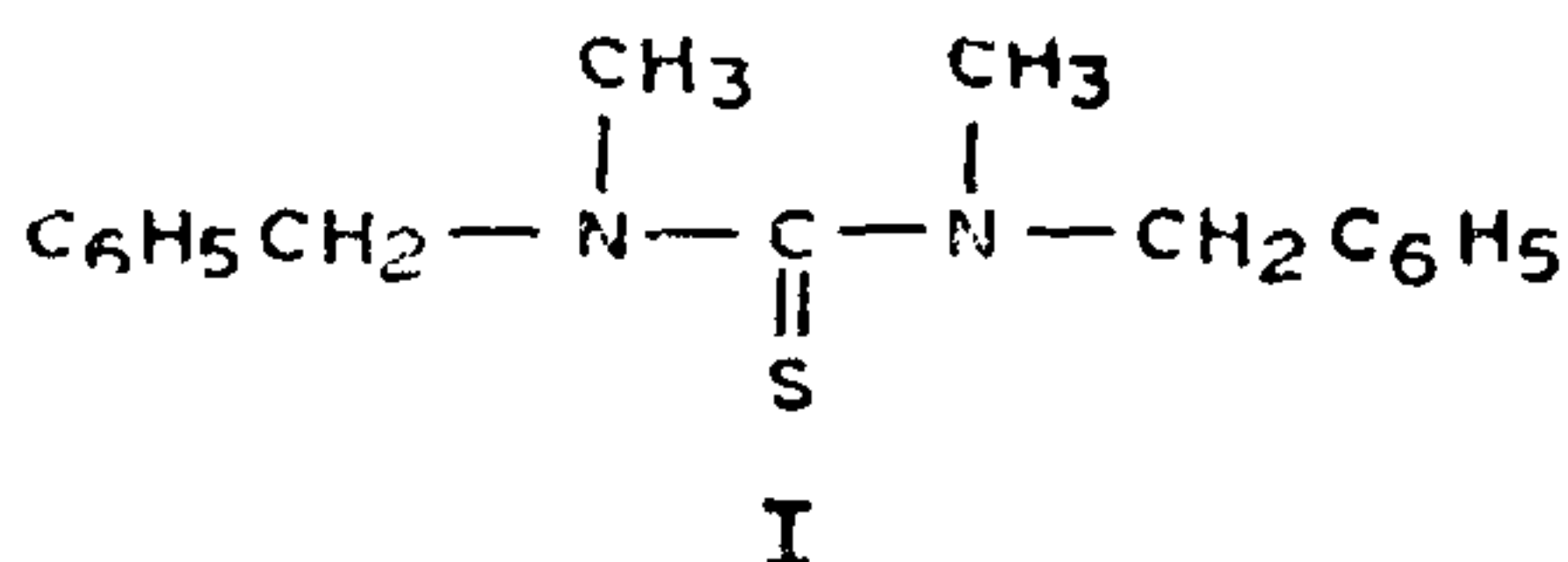


CONSTITUTION OF THE NEW THIOUREA DERIVATIVE FROM THE SEEDS OF *SALVADORA OLEOIDES*

THE seeds of *Salvadora oleoides* called 'Kha-kan' and 'Chhota pilu' in India were examined by us¹ and among the number of compounds symmetrical dibenzyl thiourea, symmetrical dibenzyl urea and a new thiourea derivative, m.p. 180-82° C were isolated. The structure of the new thiourea derivative was tentatively given as symmetrical di(*N*-methyl-*N*-styryl) thiourea. A re-examination of its analytical data and NMR spectrum, necessitates a revision of its structure as symmetrical di(*N*-methyl, *N*-benzyl) thiourea (I). The elemental analysis agrees with this formula $C_{17}H_{20}N_2S$. The compound shows only one maximum at 280 nm in the UV spectrum. The NMR spectrum in $CDCl_3$ has three sharp singlets at 7.35, 5.48 and 1.95 ppm. Integration curve shows that they correspond to 10, 4 and 6 protons respectively. It is remarkable that the most downfield signal is a singlet representing two unsubstituted phenyl groups; usually, it is a multiplet in flavonoids. But it agrees with the NMR of toluene which also shows a singlet for the phenyl group at δ 7.17 ppm². A similar feature has been recently observed in the case of dalbergi-chromene in regard to its 4 phenyl group.³ The most upfield singlet 1.25 represents two methyl groups which could be either on similar nitrogen atoms or in an aromatic nucleus. The latter is not possible because the aromatic rings are not substituted as shown by the NMR spectrum. Hence, the former possibility exists. The singlet at δ 5.48 ppm could be attributed to four protons of two benzylic methylenes linked to N. Thus, the structure of the compound would be symmetrical di (*N*-benzyl, *N*-methyl) thiourea (I). This is supported by its co-occurrence with dibenzyl urea



and thiourea. In NMR spectra, all these benzyl groups have similar values, e.g., dibenzyl thiourea has for the CH_2 a doublet centred at δ 4.81. The new thiourea derivative has not been described in the literature so far and this is the first report of its natural occurrence.

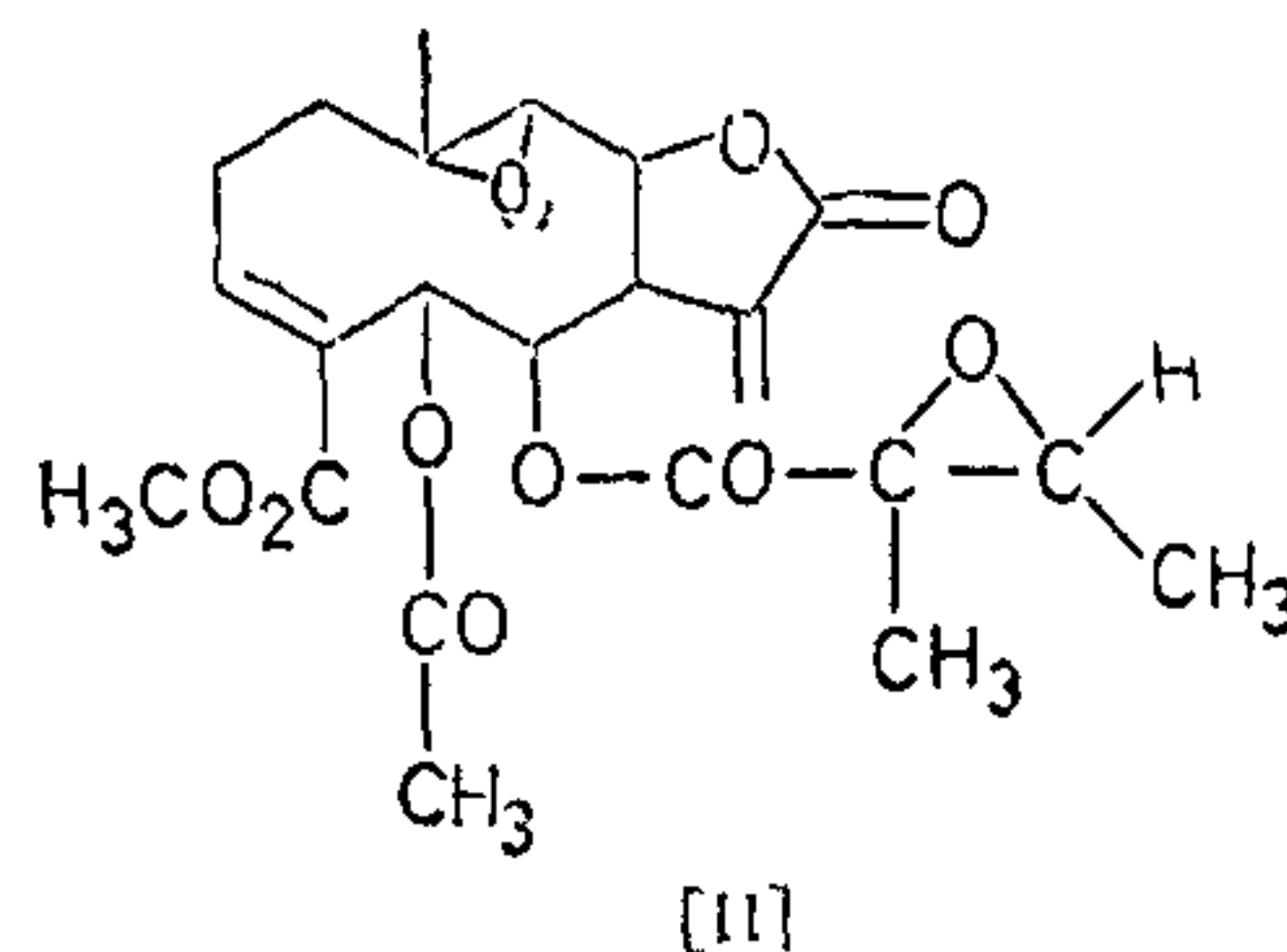
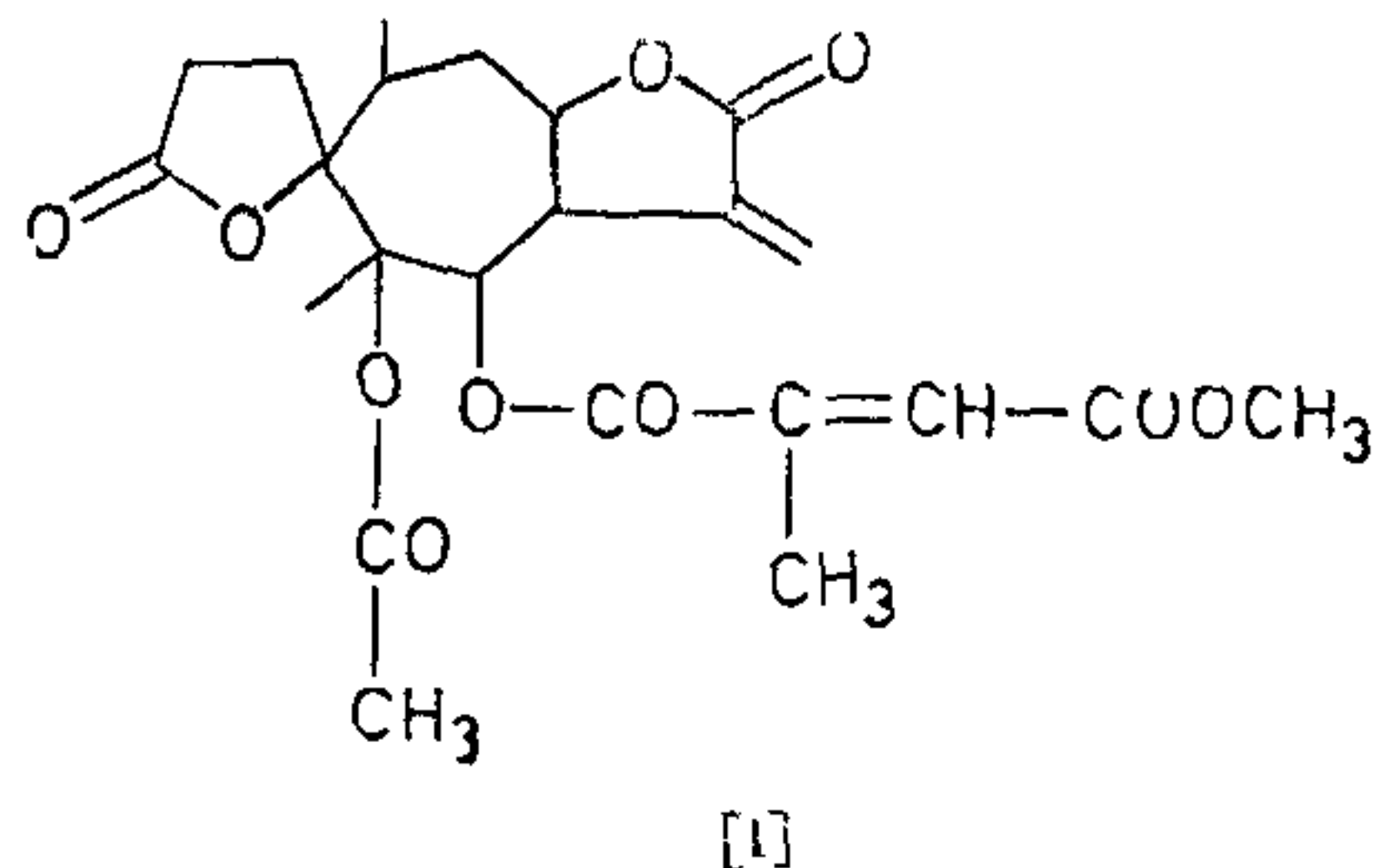
Dept. of Chemistry,
University of Delhi,
Delhi-7, February 3, 1971.

G. R. CHOPRA.
A. C. JAIN.
T. R. SESHADRI.

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STRUCTURE OF ENHYDRIN

IN our earlier communications^{1,2} we reported the isolation of enhydrin, a new sesquiterpene lactone from *Enhydra fluctuans* (Family: Compositae) and suggested a tentative structure (I) for it based on our limited observations. As the yield of the compound was low and as there were variations from batch to batch of the plant material, we had to rely mainly on the spectral data. We have subsequently isolated more of it and carried out several reactions, and on the basis of the consolidated data, propose a revised structure. The expression (II) now arrived at, seems to satisfactorily explain all the observations.



As reported earlier, enhydrin, $C_{23}H_{28}O_{10}$, m.p. 185-86° C, $[\alpha]_D^{25} -55.6^\circ$, had an $\alpha : \beta$ -unsaturated γ -lactone unit as inferred from UV, IR and NMR data. This was supported by the liberation of formaldehyde on ozonolysis. The compound showed the presence of two double bonds in quantitative perbenzoic acid titra-

tion. The presence of a carbomethoxyl and an acetoxyl groups were clear from the NMR spectra of enhydrin and of its various other derivatives.

Consideration of the molecular formula and elimination of possible alternatives strongly indicated that the compound is a sesquiterpene lactone with an acetoxyl and a carbomethoxyl groups and there should be another hydrolysable group in the form of a C_5 acyloxy function. This was also suggested by the presence of four carbonyl peaks in IR spectrum under good resolution.

Based on a series of chemical transformations, this C_5 -acyl unit has now been proved to be present in the form of an epoxytigloyl unit. The formation of a chlorohydrin on treatment with hydrochloric acid and of a vicinal diol with oxalic acid were found to involve this C_5 -acyl chain and thus there were clear evidences to show the presence of an epoxide group in the C_5 -acyl chain. This structural unit also corresponded with the appearance of a ($M^+ - 116$) peak in the mass spectrum. Alkali titration of the parent compound indicated the presence of four hydrolysable groups arising from the lactone, acetoxyl, carbomethoxyl and epoxytigloyl units. All these observations made it necessary to revise the tentative structure (I).

The above functional groups accounted for nine out of the ten oxygen atoms present in enhydrin. That the remaining oxygen was neither a free hydroxyl nor a ketone was clear from spectral data and chemical reactions. The presence in the NMR spectrum of a sharp signal at 1.71δ for three protons could be attributed to a methyl group attached to a carbon atom bearing an oxygen atom in the form of an ethereal link. This observation indicated that the tenth oxygen might be present as a second epoxide unit. This is accommodated in structure (II) most satisfactorily.

Full details of the study of enhydrin will be published elsewhere.

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March 29, 1971.

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1, 2-DIHYDROXYBENZENE-4-MONOCHLOROACETYLATE—A SELECTIVE REAGENT FOR TOTAL IRON DETERMINATION

Low concentrations of Zn, Cu (ic), Ag, Cd, Co (ic), Hg (ic), UO_2 and MoO_4 ions interfere at low concentrations ($1-20\mu g$) of iron with various colorimetric methods of iron estimation.¹⁻³ With 1, 2-dihydroxybenzene-4-monochloroacetyl, which has not been used previously for iron estimation, these ions do not interfere even when present in concentrations higher than are usually encountered in biological materials. The reagent forms a red-coloured chelate with iron in alkaline medium. It is also capable of estimating total iron without pre-oxidation or pre-reduction. The colour is stabilised with polyvinyl alcohol (PVA).

Tris 0.2 Molar was found to be the most suitable pH buffer. Strongly acid samples may be neutralized with either NaOH or NH_4OH to pH 7.0. An excess of chelating reagent results in a rapid development of colour; but it also leads to its rapid fading so that stability plateaux is very much condensed. In view of this, a ratio of one part of iron to 10 parts of chelating reagent is required. The absorbance of the iron chelate is found to be maximum at $520 m\mu$ and Beer's law is obeyed in the range of $0-50\mu g$ of iron.

Reagents

- (1) 1, 2 - dihydroxybenzene - 4 - monochloroacetyl (0.052% W/V) 0.026 g dissolved in 50 ml water at $50^\circ C$.
- (2) Polyvinyl alcohol 0.05% (W/V) in water.
- (3) 0.2 M Tris (Hydroxyl) methyl amine [2-amino 2-(Hydroxymethyl) propane-1:3 diol]—B.D.H. L.R. grade.

For iron standard, 0.07023 g ferrous ammonium sulphate (A.R.) is dissolved in 10 ml of 0.01 N HCl and made up to 100 ml. This gives $100\mu g/ml$.

Place 1 ml or less of test solution containing $10-50\mu g$ iron in a wide test-tube. Adjust the volume to 1.5 ml with glass distilled water. Add 1 ml of reagent (1). Next add 1.0 ml of PVA, reagent (2). Last, add 6.5 ml of tris, reagent (3). Mix gently and measure the absorbance in a Baush and Lomb Spectronic-20 colorimeter at $520 m\mu$ against the reagent blank. The colour is stable for an hour.

Figure 1 gives the absorbance of the chelate formed with a constant amount of iron and varying amounts of chelating reagent. It is