

A STUDY OF THE CHEMICAL COMPONENTS OF *ENHYDRA FLUCTUANS*

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*ENHYDRA FLUCTUANS* is a marshy herb belonging to the family *Compositæ* (tribe, *Helianthæ*) and has been used in Indian medicine in the treatment of skin diseases, nervous ailments and as a laxative.<sup>1</sup> Botanically and in its use it is closely allied to *Eclipta alba* which we have analysed recently in detail.<sup>2</sup> *Enhydra fluctuans* was examined earlier by Chakravarti and Dutta<sup>3</sup> who isolated stigmasterol. In our experiments we found that there is considerable variation from batch to batch of the plant material and it is not always available in the fresh condition. We now make a brief preliminary report of some special observations on the components of this plant.

From a petroleum ether extract of one of the batches collected in March, 1967, a very small yield of a lactone could be obtained merely on cooling the hot extract. It does not agree with any of the *Compositæ* lactones reported so far and has therefore been named 'enhydrin'. As the amount available was small only a preliminary study, mainly spectral, has so far been possible. It crystallised from alcohol as colourless needles, m.p. 185-86°. The elemental analysis and molecular weight determination by mass spectrum (464) agreed with the molecular formula  $C_{23}H_{25}O_{10}$  and it contained one methoxyl group (Found:  $OCH_3$ , 6.0%;  $C_{22}H_{25}O_9$ ,  $OCH_3$  requires  $OCH_3$ , 6.7%). In its solubility and TLC behaviour and colour reaction with concentrated sulphuric acid (golden-yellow turning dark brown on gentle heating) enhydrin exhibited similarities with other sesquiterpene lactones of *Compositæ* such as coronopilin,<sup>4</sup> ivaxillarin<sup>5</sup> (samples kindly supplied by Prof. W. Herz) and helenalin.<sup>6</sup> Its U.V. spectrum (in ethanol) had a strong end absorption in the region 220-240 m $\mu$  but no distinct maximum (cf. xanthinin<sup>7</sup>). The IR spectrum (nujol) had three frequencies in the carbonyl region at 1790, 1775 and 1730  $cm^{-1}$  (in KBr an inflexion at 1750  $cm^{-1}$  was also noticeable) which were suggestive of the presence of saturated  $\gamma$ -lactone as in psilostachyin,<sup>8</sup>  $\alpha$ - $\beta$  unsaturated- $\gamma$ -lactone and ester groups; the absorption at 1650  $cm^{-1}$  is attributable to an exocyclic double-bond conjugated with the  $\gamma$ -lactone, for which there is analogy in the observations of Herz and co-workers.<sup>5,10</sup> The weak, broad absorption at 3,650  $cm^{-1}$  could be

due to a hydroxyl which must be tertiary since enhydrin failed to undergo acetylation. The presence of the exocyclic double-bond was further supported by absorptions at 3,100 and 1,420  $cm^{-1}$  and confirmed by the characteristic pair of doublets (one proton each) at  $\delta$  5.79 and 6.32,  $J=3$  in the NMR spectrum (in benzene).

The NMR spectrum was run at 60 MC both in benzene and in chloroform but as the solubility of enhydrin in benzene was greater, the spectrum in that solvent was better resolved. Therefore the signal positions given in Table I are those observed in benzene solution; those of chloroform solution are given in brackets. The total proton count was 28.

TABLE I  
NMR spectrum of enhydrin

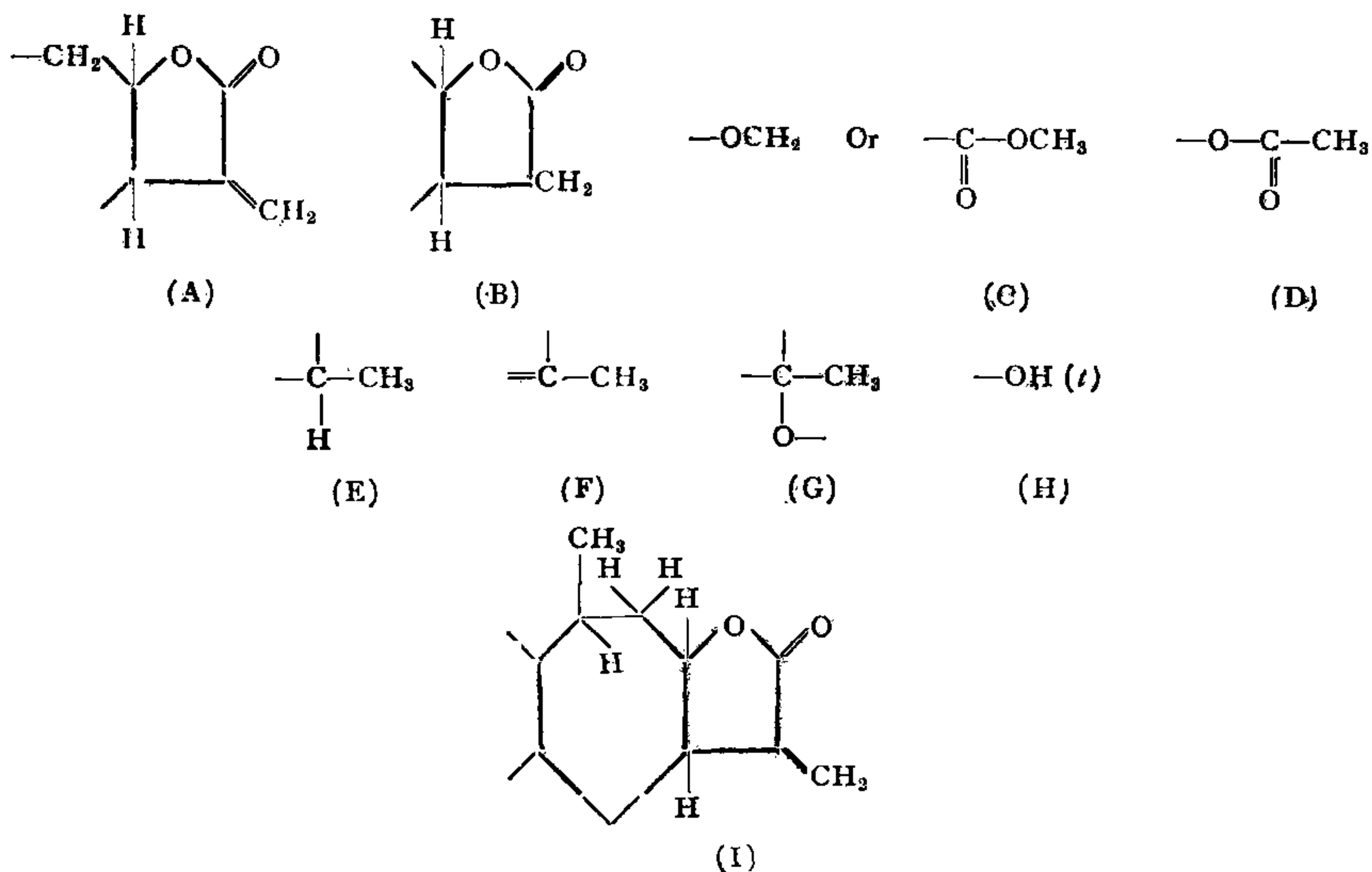
Signal position in ppm	No. of protons	Assignment
1.05 (1.17)d ( $J=6$ )	3	A secondary methyl (E)
1.35 (1.45)	3	A tertiary methyl on a carbon carrying an oxygen function (G)
1.62 (1.72)	3	A tertiary methyl on a double bond (F)
1.67 (2.07)	3	Acetoxy (D)
1.8-3.1 (A group of complex signals)	7	..
3.57 (3.86)	3	Methoxyl or carbomethoxyl (C)
Ca 3.96	1	..
4.34 ct	1	Lactonic hydrogen (A)
5.79 d ( $J=3$ )	1	Exocyclic methylene conjugated with a lactone carbonyl (A)
6.32 d ( $J=3$ )	1	
5.92	1	..
6.17	1	May be a hydroxyl proton (signal position solvent dependent)

'd' denotes doublet, 'c' complex and 'ct' complex triplet.

The signals at 1.67(2.07) and 3.57(3.86) were markedly influenced by the nature of the solvent, benzene causing upfield shifts expected for the assignments given.<sup>9</sup> The characteristic complex triplet at 4.34 could be attributed either to the lactonic proton, i.e., the one adjacent to the oxygen of the unsaturated  $\gamma$ -lactone as in gaillardin,<sup>10</sup> calocephalin<sup>11</sup> and related compounds (part structure I) or to a proton in a similar environment. The former alternative is more likely since no signals

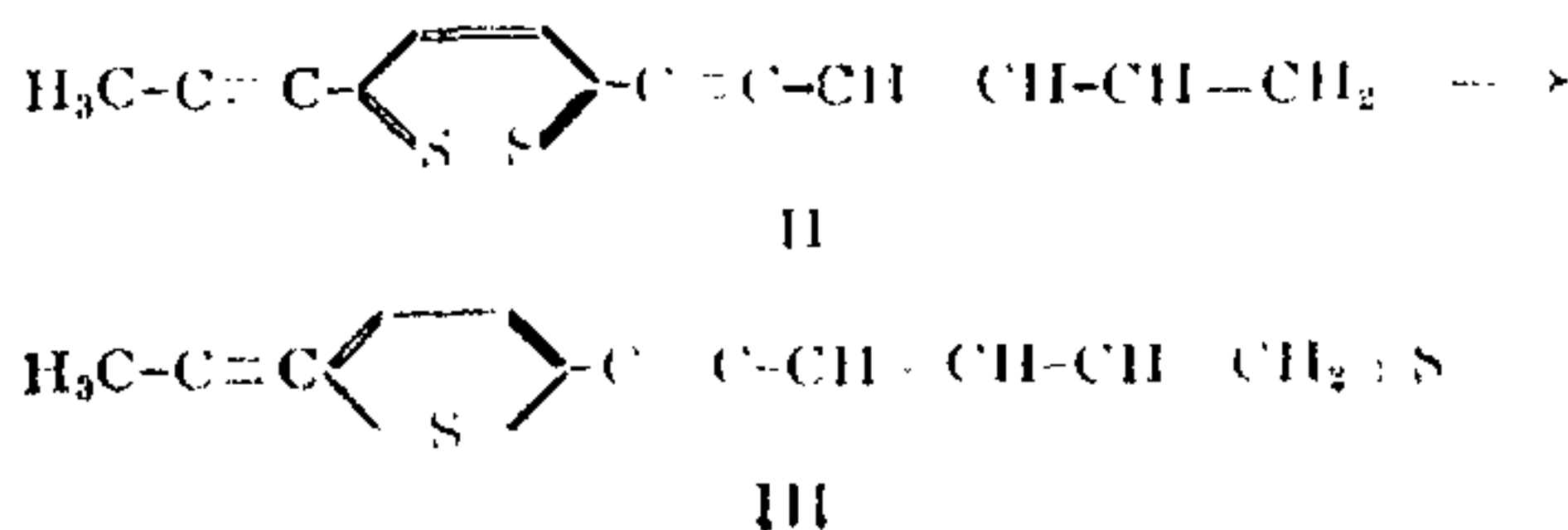
otherwise attributable to this lactonic proton could be detected. On the basis of these data the groups A to H are recognizable in enhydrin and the part structure (I) may be present in it also

262-264 m $\mu$ . It was identified as elemental sulphur, the data agreeing with those for monoclinic. Obviously, it has been derived from a sulphur compound present in the plant. Bohlmann and Kleine<sup>12</sup> have recorded the isolation



A study of the mass spectrum supports some of the features mentioned above. Besides the molecular ion peak at m/e 464, some other prominent peaks appeared at m/e 446(M-18), 433(M-31), 405(M-59) and 348(M-116). The last-mentioned ion is the second most abundant, the base peak appearing at m/e 58. The M-18 peak supports the presence of a hydroxyl group and those at 433 and 405 support the carbomethoxyl. Further studies on the structure of enhydrin are in progress.

plates and prisms, m.p. 118-20°;  $\lambda_{\text{max}}^{\text{cyclohexane}}$  of dark red oily compounds such as (II) accompanying the polyacetylenes and related thiophenes from extracts of some plants of *Helianthæ* and *Heleniæ*. These compounds are unstable and decompose during chromatography or even in solution in course of time, yielding thiophenes such as (III) and elemental sulphur. It is probable that the sulphur of *E. fluctuans* might also have been derived from similar compounds. The accompanying orange-red oil mentioned above showed on TLC three spots exhibiting blue fluorescence under UV light much like the acetylenic thiophenes. Examination of the UV spectra of these components after TLC separation showed that they were similar to the spectra of acetylenic thiophenes though no definite identifications were possible.



The petroleum ether extract, after removal of enhydrin, was further concentrated when a second solid separated. On TLC it gave a number of spots, two of which exhibited bright blue fluorescence under UV light. The brighter of these was eluted and a spectrum taken,  $\lambda_{\text{max}}^{\text{EtOH}}$  255, 350-355 m $\mu$ . The amount, however, was too little for further characterisation. The filtrate on concentration and chromatography over alumina yielded an orange-red petroleum ether eluate which gave a pale yellow crystalline solid and an orange-red oil. The solid answered the Lassaigne test for sulphur and crystallised from alcohol as pale yellow

A petroleum ether extract of another batch of the plant collected during the summer of 1967 did not yield enhydrin but gave elemental sulphur and the orange-red oil besides a good yield of a long chain aliphatic ester (m.p. 84-86°). A subsequent chloroform extract, did, however, contain enhydrin as shown by TLC but it could not be isolated in a pure state.

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## RADIOCARBON DATES OF KALIBANGAN SAMPLES

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**K**ALIBANGAN, the well-known Harappan site on the river Ghaggar, has been extensively excavated (Ghosh, 1960-63) by B. B. Lal and B. K. Thapar under the aegis of the Archaeological Survey of India. The site was extensively sampled for both Kalibangan I and II periods. The  $C^{14}$  dates of the samples collected from this site and a brief discussion thereof are presented in this article.

All samples are cleaned manually first to get rid of extraneous matter including visible rootlets, and then treated with dilute HCl to remove soluble carbonates. Wherever possible, NaOH pretreatment was also given to remove any humic acid present. Samples are counted in the form of methane in gas proportional counters. For modern reference standard 95% activity of N.B.S. oxalic acid was used. Processing procedures have been described in detail earlier (Agrawal et al., 1965).

All dates are given in years B. P. The first date is based on radiocarbon half-life of 5568 yrs.; the second, within brackets, is based on the value of 5730 yrs.

### DISCUSSION

Kalibangan provided a very rich site for extensive sampling. A sizable number of samples was dated not only to determine the time-spreads of the two cultures, but also to study the internal consistency and factors

responsible for divergences, if any. As the excavations were scientifically controlled, any ambiguity due to stratification errors could largely be avoided. The only errors which could arise at this site were those due to humic acid and such other factors.

The Kalibangan Period I dates are all consistent, except for TF-240. Due to the presence of structures above, good levels with proper soil-cover generally could not be tapped for sampling the Period I sequence. Most of the samples are derived from the periphery of the mound. Thus humus contamination coupled with the inherent errors of the order of  $\pm 100$  yrs. can easily magnify such short-time brackets; the spread therefore could be smaller but in no case larger.

Kalibangan has by now been extensively dated (Agrawal and Kusumgar, 1966). For Kalibangan II (Harappan) a consistent sequence of dates exists for the early and middle phases. This helps in selecting the meaningful dates from the scatter of late phase.

We would discuss below the factors which could affect the dates at Kalibangan site.

The role of soil-cover in preserving the samples against contamination has been recognized. Sites that have been deserted thousands of years before, like Kalibangan, get humic acid from organic decay right up to the time