

on root exudations and is comparable to observations made under field conditions. The implications of these results can be better understood if the effects of the chemical directly or indirectly on plant metabolism are studied alongside rhizosphere microbial ecology.

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## CHEMICAL COMPONENTS OF *ENHYDRA FLUCTUANS*—II

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IN an earlier communication,<sup>1</sup> we reported the isolation and preliminary study of a new sesquiterpene lactone, named enhydrin from a petroleum ether extract of *Enhydra fluctuans*, a plant used in India as drug. We now report some further observations on enhydrin and on another new component of the plant. Our experience is that the yields of these compounds are poor and vary from batch to batch of the material.

As stated earlier,<sup>1</sup> enhydrin has the molecular formula  $C_{23}H_{28}O_{10}$ . The presence of  $\alpha:\beta$ -unsaturated  $\gamma$ -lactone inferred earlier from i.r. and nmr spectral data is further supported by the following observations. Enhydrin answers the Legal test (cf. Mexicanin A) and reduces Tollen's reagent. Using a better instrument, a careful recording of its uv spectrum in ethanol below  $220 m\mu$  revealed an intense absorption maximum at  $214 m\mu$  ( $\epsilon = 17,200$ ). In addition, another maximum of much less intensity was observed at  $270 m\mu$  ( $\epsilon = 6,570$ ).

The presence of an acetoxyl group was confirmed by an acetoxyl group estimation. A quantitative hydrogenation using a Pd/C catalyst showed the presence of at least one double bond in enhydrin. But the other data (analytical, uv and nmr) indicate the presence of one more double bond in enhydrin and this does not probably suffer easy hydrogenation.

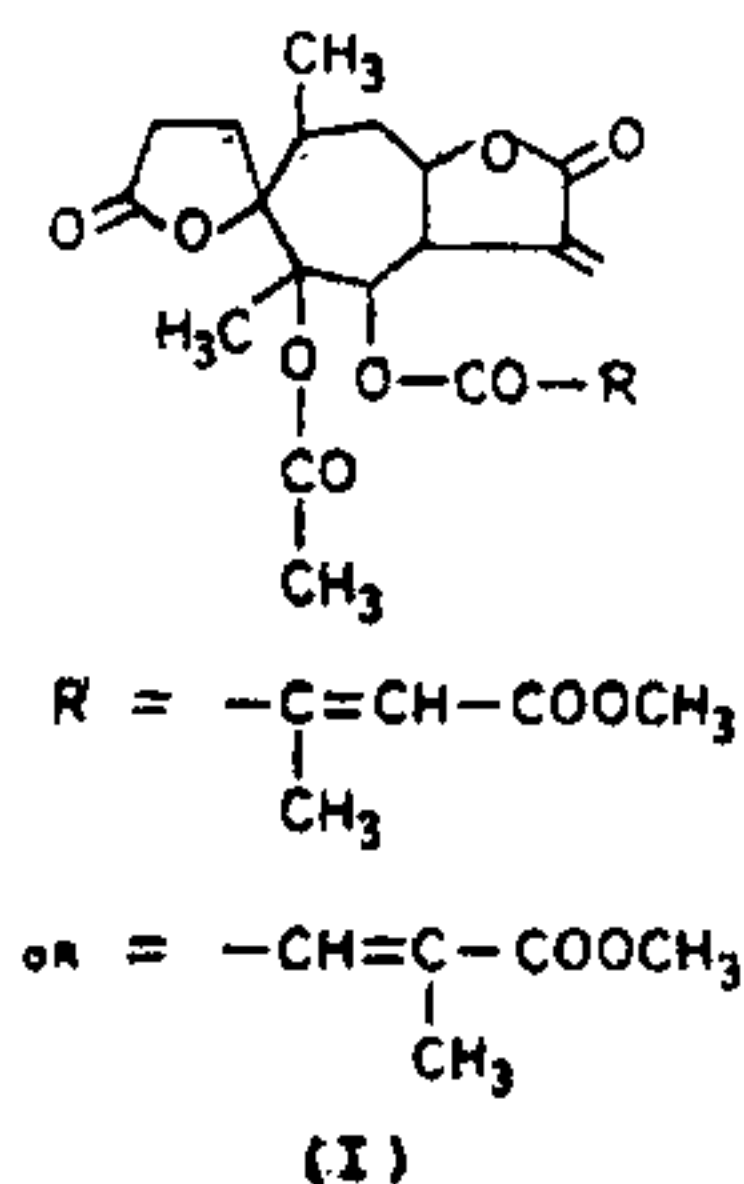
The nmr spectrum of the product of hydrogenation differed from that of enhydrin in the following respects. The signals due to the exocyclic methylene protons had disappeared in the hydrogenated product and instead the doublet signal at  $\delta$  1.25 p.p.m., due to secondary methyl integrated for 6 protons as against 3 in enhydrin. There was no change in the number or position of the other methyl groups. Obviously, only the  $\alpha:\beta$ -unsaturated lactone ring had suffered hydrogenation.

Earlier,<sup>1</sup> we had inferred the presence of a tertiary hydroxyl group based on the observations that the i.r. spectrum had a weak broad absorption at  $3650 cm^{-1}$  and the mass spectrum had a small peak at  $m/e$  446, attributable to M-18. However, a micro estimation showed the absence of any active hydrogens in enhydrin. In order to check this point and also for the following reason, the mass spectrum of the compound was re-taken. The earlier record of the base peak at  $m/e$  58 was an error and it was actually at  $m/e$  78 which could have been due to benzene of crystallisation as the sample had been re-crystallised from that solvent. On repetition of the mass spectrum using a thoroughly dried sample, we found this peak to be indeed absent; now the base peak is the one at  $m/e$  348. A prominent peak in the new spectrum was at  $m/e$  404 (M-60) which was more intense than  $m/e$  405 reported earlier. This peak could be explained as due to the loss of acetoxyl as acetic acid. Apart from

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these differences, the two spectra were alike; thus in both the molecular ion peak appeared at  $m/e$  464. (We are not able to understand how the peak at  $m/e$  446 arises.) Since the spectral and analytical data had previously indicated the presence of two  $\gamma$ -lactone groups, one saturated and the other  $\alpha$ : $\beta$ -unsaturated, the base peak at  $m/e$  348 might be due to a M-60-28-28 fragment. The peaks at  $m/e$  91 and 105 may be significant as Geissman and Mukherjee<sup>2</sup> have noted these peaks in the mass spectra of all the sesquiterpenoid lactones examined by them.

Further, there were indications of the presence of a second acyloxy group besides the acetoxyl in enhydrin. The presence of such a fraction was indicated by the molecular formula assuming a sesquiterpene skeleton and by the position and high  $\epsilon$  value of the uv absorption maximum at  $214 m\mu$ . This also suggested the probable presence of another chromophore such as  $\alpha$ : $\beta$ -unsaturated ester besides the unsaturated  $\gamma$ -lactone. If one such was present it would probably be a  $C_5$  or a  $C_6$  unsaturated acyloxy group. However, the i.r. spectrum (no band below  $1730 cm^{-1}$  in the carbonyl region) did not favour the presence of an  $\alpha$ : $\beta$ -unsaturated ester group of the tigloyl or allied type found in some sesquiterpene lactones. The nmr data also ruled out such units. These results could only be explained if the acid part of the acyloxy group consisted of an  $\alpha$ : $\beta$ -unsaturated dicarboxylic system, probably as its mono-methyl ester. In such a case, the  $\alpha$ : $\beta$ -unsaturated ester carbonyl band will shift to a higher frequency due to opposing conjugation.<sup>3</sup> A probable tentative structure for enhydrin would then be (I).



In order to verify the above conjecture, an alkaline hydrolysis was attempted on enhydrin.

Treatment with dilute methanolic alkali at room temperature gave a mixture of products, none of which was identical with the starting material. However, attempts to fractionate this mixture into acidic and neutral fractions were not successful and it has not been possible so far to isolate and characterise any of the products.

In the earlier communication,<sup>1</sup> we had reported our experience with regard to the variation in contents of *E. fluctuans* from batch to batch. A better yield of pure enhydrin is obtained from the leaves of the plant, whereas when the leaf-content of the whole plant is less, a complex mixture is obtained from which enhydrin is isolated only with difficulty. Since it is a marshy plant available near Calcutta, we have been able to get only the air-dried whole plant and a certain amount of variation in contents and yield from batch to batch could not be avoided.

From one of the batches we isolated a minor component which appeared along with enhydrin in the petroleum ether extract. However, this compound was insoluble even in hot benzene in which enhydrin is freely soluble and thus the two could be easily separated by crystallisation. As the quantity available was very small (less than 50 mg.) only a brief preliminary study has so far been possible. It has a melting point of  $272-77^\circ C$ , and the elemental analysis and molecular weight determination (mass spectrum, 320) agree with the molecular formula  $C_{20}H_{32}O_3$ . In t.l.c. it has a lower  $R_f$  than enhydrin and its uv spectrum in ethanol exhibits only end absorption above  $210 m\mu$  ( $\epsilon$  at 210, 3840). The i.r. spectrum has frequencies at  $3650 cm^{-1}$  and  $1718 cm^{-1}$ . The former may be attributed to the presence of a hydroxyl function. This is supported by the formation of an acetate with acetic anhydride and pyridine and also by the appearance of a prominent peak at  $m/e$  302 (M-18). The absorption at  $1718 cm^{-1}$  is very sharp and there is no indication of any unsaturation. This suggests that the carbonyl group in this compound may either be a lactone (J) or a carboxyl group. The mass spectrum of this compound has other prominent peaks at  $m/e$  262, 147, 135, 121, 109, 105, 94, 91, 81, 79, 55 and 43. The base peak is at  $m/e$  43.

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