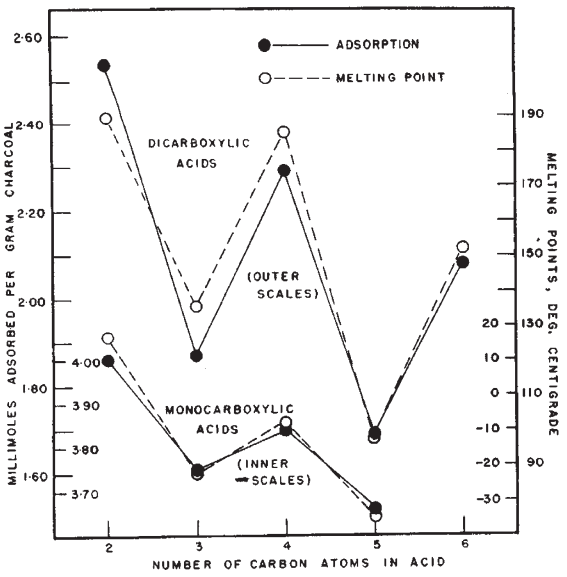

Fig. 1

between the alternations in melting points and adsorptions. Thus it would appear that the saturated adsorbed film is in a highly oriented state such as in crystalline solids.

We believe that an explanation for the alternation in the case of adsorption must be looked for in differences in lateral packing rather than in the lengths of the chains[3]. Moreover, our observations tend to refute the suggestion that adsorption from binary mixtures on porous adsorbents is always by a pore-filling mechanism[4]. Rather, they strongly suggest that adsorption of the aliphatic acids from aqueous solutions on porous carbons is in unimolecular layers.

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Identification of an Artefact on Chromatograms of the Keto-acid 2,4-Dinitrophenylhydrazones

DURING a study of plant keto-acids carried out by one of us (G. H. N. T.) under the direction of Prof. F. C. Steward at Cornell University, an unknown substance, hereafter designated compound I, was found in the keto-acid fraction of all plant tissues examined[1]. This substance was isolated in crystalline form (sint. 206°, decomp. 214°) from the keto-acid dinitrophenylhydrazone fraction of *Mentha piperita* leaves by elution from an alumina column with 1 per cent sodium carbonate. Its position on chromatograms was similar to that of the slower-moving spot of pyruvic acid dinitrophenylhydrazone (Fig. 1, ref. 1). The pyruvic acid spot developed a chocolate colour when sprayed with alkali, whereas compound I gave a yellow colour. On hydrogenation, compound I yielded a ninhydrin-reactive substance (compound II) which was chromatographically similar to γ-aminobutyric acid (Fig. 2, ref. 1). The infra-red absorption spectrum of compound I did not display the carboxyl peak characteristic of α-keto-acid dinitrophenylhydrazoness.

We have utilized the same procedures[1] for a study of the relation between the keto-acids and the fixation of carbon-14 dioxide in leaves. Compound I consistently recurred on chromatograms of the keto-acid dinitrophenylhydrazoness. Although under favourable conditions radiocarbon was incorporated by the leaf into most of the recognized keto-acids, it never entered compound I. We therefore suspected that it might be an artefact.

When the reagent (2,4-dinitrophenylhydrazine) was dissolved in ethyl acetate and extracted with alkali in the manner used in the keto-acid method, a brown gum was recovered from the alkaline extract. Chromatograms of this gum showed two main spots: one, a fast-moving brown spot; the other, identical in position with compound I. Alkali-extracted and recrystallized 2,4-dinitrophenylhydrazine still gave these substances on a second extraction with alkali.

A survey of the literature showed that an alkali-soluble compound, 1-hydroxy-6-nitro-1 : 2 : 3 benzotriazole, is obtained on treating 2,4-dinitrophenylhydrazine with alkali. Accordingly, some of this compound was prepared by refluxing 2,4-dinitrophenylhydrazine with hydrazine hydrate[2], and its physical and chemical properties were compared with those of compound I isolated from *Mentha* leaves. The two substances were found to be identical with respect to infra-red spectra, melting characteristics and chromatographic co-ordinates.

This finding has an important bearing on the current use of 2,4-dinitrophenylhydrazine as a reagent for the detection of keto-acids. The artefact may interfere on one-directional paper chromatograms of keto-acid dinitrophenylhydrazones because its position overlaps that of pyruvic acid dinitrophenylhydrazone. The effect may, however, be minimized by avoiding an excess of reagent. In addition, the artefact may be partially separated from the slower-moving spot of pyruvic acid dinitrophenylhydrazone with certain solvents as shown in Table 1.

Table 1

	R_F values		
Solvent	*A*	*B*	*C*
1-Hydroxy-6-nitro-1 : 2 : 3 benzotriazole	0·56	0·43	0·81
Pyruvic acid dinitrophenylhydrazone (slower-moving spot)	0·50	0·40	0·84

A, *t*-amyl alcohol/ethanol/water (ammonia vapour), 9/1/2.
B, *t*-amyl alcohol/*n*-propanol/ammonium hydroxide, 13/1/6.
C, 0·5 per cent sodium carbonate.

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