which had also been given 3 mg of ascorbic acid, recovered slowly.

According to the results presented, guinea-pigs are on the average able to utilize about 10 to 15 % of the ascorbic acid bound in ascorbigen. The observation made in our first communication that 26 mg of ascorbigen has a curative effect on a scorbutic guinea-pig, was thus confirmed

When using cabbage as a fresh vegetable, the formation of ascorbigen is of no practical importance. No decrease in ascorbic acid was found after normal chewing which lasted for 10-13 sec.

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- Kiesvaara, M. and Virtanen, A. I. Acta Chem. Scand. 16 (1962) 510.
- Virtanen, A. I. and Kiesvaara, M. Acta Chem. Scand. 17 (1963) 848.
- Harris, L. J. and Olliver, M. Biochem. J. 36 (1942) 155.

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The Separation of Pimaric and Isopimaric Acids by Partition Chromatography at Low Temperatures

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Partition chromatography employing silicic acid as the carrier, a mixture of 2-aminopyridine and furfuryl alcohol as the stationary phase, and isooctane as eluent has been successful since 1955 in the analysis of rosin acid mixtures such as oleorosins, gum rosin, wood rosin, tall oil rosin acids, and catalytically modified rosin acid mixtures (Ref. 1-2, and references cited in Ref. 2). Although tetrahydroabietic, dihydroabietic, dehydroabietic, neoabietic, palustric, and abietic-levopimaric acids can be separated from one another satisfacto-

rily by this method, pimaric and isopimaric acids are eluted together. In work on the analysis and synthesis of rosin acids and their mixtures, it would be of value to have a partition-chromatographic method which would permit the separation and analysis of the last two acids. We have found that by carrying out the partition chromatography at low temperatures, it is possible to alter the elution behaviour of the two acids sufficiently to effect their separation. The following results relate to the partition chromatography of an artificial mixture of the two pimaric acids at temperatures between +1 and $+5^{\circ}\mathrm{C}$.

The chromatographic column, the re-

servoir for the eluent and the tubes connecting them were kept at the temperature in question by means of a cooling medium consisting of a mixture of ethylene glycol and water which was circulated from a cryostat in which the reservoir for the eluent was immersed. The column with a inner diameter of 11 mm was filled with Mallincrodt's silicic acid (analytical reagent, 100 mesh) that had been treated with a

mixture of 2-aminopyridine (m.p. $51-56^{\circ}$ C; British Drug Houses Ltd. recrystallized from ligroin), and technical furfural alcohol (British Drug Houses Ltd.) that had been shaken with sodium hydroxide pellets and redistilled at 15 mm Hg. 9 ml of this stationary phase was mixed with 15 g of the silicic acid. The isooctane $(n_D^{20} = 1.390-1.392)$ employed as eluent was a product of the British Drug Houses Ltd. It was purified by passing it through a column of aluminium oxide (for chromatography according to Brockmann, E. Merck AG) and then distilling it. This eluent was saturated with the stationary phase. A Par-

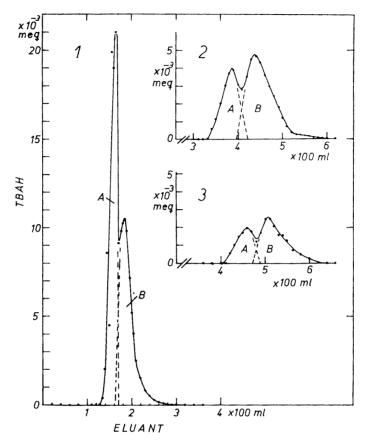
eluent through the column.

The eluate was collected in 5-ml fractions by means of a siphon. The acids in the eluate fractions were determined by titration with 0.01 N tetrabutylammonium hydroxide (TBAH) in isooctane employing thymol blue as indicator.

valux micropump (The Distillers Company Ltd, England) was employed to move the

The results obtained in three chromatographic runs are plotted in Figs. 1—3. It will be noted that isopimaric acid took a longer time to pass through the column than pimaric acid. The separation factor, the ratio of the peak volumes, varied in these cases from 1.10 to 1.12.

The idea that led to the preceding study was an observation made by one of us in 1954³ that some of the properties, e.g.



Figs. 1-3. Chromatograms for mixtures of pimaric (DP) and isopimaric (IDP) acids obtained by partition chromatography at low temperatures. Eluent: isooctane. Fig. 1: 17 mg DP + 15.7 mg IDP; stationary phase 2-aminopyridine — furfuryl alcohol 1:1; flow rate 11 cm h⁻¹cm⁻²; temperature + 3 — + 5°C. Fig. 2: 10 mg DP + 15.2 mg IDP; stationary phase 2-aminopyridine—furfuryl alcohol 1:1; flow rate 14 cm h⁻¹cm⁻²; temperature + 1 — + 4.5°C. Fig. 3: 6.4 mg DP + 11 mg IDP; stationary phase 2-aminopyridine—furfuryl alcohol 1:3; flow rate 17 cm h⁻¹cm⁻²; temperature + 1 — + 1.5°C. TBAH = tetrabutylammonium hydroxide. Peaks A refer to pimaric acid and peaks B to isopimaric acid.

the areas taken up by molecules in a monolayer on an aqueous substrate (studied with a surface balance), varied more with temperature in the case of isopimaric acid (IDP) than in the case of pimaric acid (DP). The area per molecule was the same for both acids at 20° C, but smaller for isopimaric acid at $+4^{\circ}$ C. It was then concluded that the difference in area must be due to a (quasi-)equatorial orientation of the vinyl group at C-7 in isopimaric acid and a (quasi-)axial orientation in pimaric

acid. This has been confirmed by other methods of investigation during the years 1955—1959 (Ref.⁴⁻⁶ and references cited in these). It was furthermore shown in these studies that also the configurations at carbon C-13 are different in these two pimaric acids.* As the different orientations

^{*} The configurations at C-7 and C-13 in sandaracopimaric acid (= cryptopimaric acid⁷) were also clarified. For additional proof of the structures of pimaric acids, see Refs.⁸⁻¹⁰

of the vinyl groups become evident in the monolayer properties of the pimaric acids, it was thought possible that the structural differences would also lead to differences in elution behaviour in chromatography if the conditions were suitably chosen. It seemed probable that if the elution rates were different, it would be likely that the molecule (IDP) whose motion is retarded more at low temperatures would be also more strongly adsorbed on a column than the other molecule (DP). This proved to be the case.

Temperature regulation is often employed in gas chromatography to improve separations and this effect has also been proposed on the basis of theoretical considerations, but this possibility has seldom been taken advantage of in partition chromatography (see, e.g. Ref. 11). As the results presented above show, different temperatures might be used more often than has been done previously when carrying out partition-chromatographic separations and analyses.

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- Loeblich, V. M., Baldwin, D. E. and Lawrence, R. V. J. Am. Chem. Soc. 77 (1955) 2823.
- Bruun, H. H. and Gåsland, S. Acta Acad. Aboensis, Math. Phys. 22 (1960) No. 1.
- 3. Bruun, H. H. Ibid. 19 (1954) No. 3.
- Bruun, H. H., Ryhage, R. and Stenhagen, E. Acta Chem. Scand. 12 (1958) 789.
- Bruun, H. H., Fischmeister, I. and Stenhagen, E. Acta Chem. Scand. 13 (1959) 379.
- Bruun, H. H. and Stenhagen, E. Acta Chem. Scand. 13 (1959) 832.
- Arya, V. P., Enzell, C. and Erdtman, H. Acta Chem. Scand. 15 (1961) 682.
- Edwards, O. E. and Howe, R. Can. J. Chem. 37 (1959) 760.
- 9. Ireland, R. E. and Schiess, P. W. Tetrahedron Letters 24 (1960) No. 18, p. 37.
- Milue, G. W. A. and Smith, H. Chem. Ind. (London) 1961 1307.
- 11. Heftmann, E. (Ed.) Chromatography, New York 1961, p. 141.

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Nicotinamide-Nucleotide Coenzymes or Nicotiniumamide-Nucleotide Coenzymes?

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Efforts to solve long-standing controversies over the nomenclature of so called pyridine-nucleotide coenzymes have led to a recent recommendation to call this group of coenzymes nicotinamide-nucleotide coenzymes. Although the recommended nomenclature indicates the general structure of these coenzymes more adequately than before, an apparently minute but actually fundamental short-coming observed in the previous nomenclature is still uncorrected in this one.

1-Methyl-3-carbamyl pyridinium iodide (I) has been called N¹-methyl nicotinamide iodide, nicotinamide methiodide, or even N-methyl nicotinamide. Although it is closely related to nicotinamide (II), this compound is quite different from nicotinamide in chemical reactivity. These conventional names fail to indicate the presence of a quaternary nitrogen in the compound. The last name, moreover, is definitely incorrect. It is the name of another compound, 3-(N-methylcarbamyl)-pyridine (III).

Radical (IV) is the functional group of the oxidized form of pyridine-nucleotide coenzymes. Upon reduction, this radical becomes radical (V), which subsequently changes to radical (VI) upon releasing a hydrogen ion. If a compound with radical (IV) is called a nicotinamide compound, as is recommended, a compound with radical (V) must be called a dihydronicotinamide compound. Then, what should one name a compound with radical (VI)? The chemically proper name of radical (VI) is actually dihydronicotinamide radical. This dilemma comes from inadequately calling 3-carbamyl-pyridinium radical (IV) nicotinamide radical.

I would like to propose radicals (IV) and (V) to be called *nicotiniumamide* and *dihydronicotiniumamide* radicals, respectively, from the following reasons: (A) the presence of the quaternary nitrogen can be clearly indicated, (B) the chemical changes involved in oxidation-reduction and addition of these radicals can be