

Tab. II. Kuhn-Roth-Abbau der Isovaleriansäure. Messfehler  $\pm 2\%$ 

	ipm/mMol	% Aktivität gefunden	erwartet
Isovaleriansäure	4740	100	
Essigsäure *	1810	38	40

\* als *p*-Nitrobenzylester.

## Flavonoid Sophorosides

Sophorose (glucosyl(1 $\rightarrow$ 2)- $\beta$ -glucose) was first isolated<sup>1</sup> from the unripe pods of *Sophora japonica*, in which it occurs combined with kampferol as the 3-sophoroside. It was later found as the disaccharide unit of stevioside, a very sweet glycoside occurring in the leaves of *Stevia rebaudiana*<sup>2</sup>. Its structure has been confirmed by at least two syntheses<sup>3,4</sup> but no other reports of its natural occurrence have appeared<sup>5</sup>. Recent studies of the sugars attached to the anthocyanins and flavones isolated from many plant sources now show that sophorose is commonly associated in nature with these pigments.

In the anthocyanin series, five pigments containing sophorose have been found. They are the 3-sophorosides and the 5-glucoside 3-sophorosides of pelargonidin and cyanidin and the 7-glucoside 3-sophoroside of pelargonidin. One or other of these pigments has been identified in the following plants: *Brassica oleracea*, var. *rubra* (leaves), five species of *Papaver* (petals), *Phaseolus multiflorus* (flowers), *Raphanus sativus* (roots), *Tropaeolum majus* (petals) and three species of *Watsonia* (petals). The occurrence of cyanidin 3-sophoroside in *Papaver rhoeas* requires special mention, because this pigment (called mecocyanin) was originally thought to be the 3-gentiobioside<sup>6</sup> and a preliminary reinvestigation<sup>7</sup> suggested that it might be the 3-cellobioside. More detailed studies, involving electrophoretic and chromatographic comparison of its sugar with all the eight known glucosyl-glucoses, now show that it is the 3-sophoroside. It should also be mentioned that the cyanidin pigment occurring in acylated form in red cabbage, now identified as the 5-glucoside 3-sophoroside, was earlier reported to be a 3-triglucoside<sup>8</sup>. Sophorose was isolated from these pigments in excellent yield by oxidizing away the anthocyanidins to which it was attached with  $H_2O_2$ , as first described by KARRER<sup>9</sup> and later modified by CHANDLER and HARPER<sup>10</sup>. Details of structural studies on the anthocyanidin sophorosides will be described elsewhere<sup>11</sup>.

In the flavone series, at least ten pigments containing sophorose are now known. Two of these, the 3-sophorosides of kampferol and quercetin, appear to occur widely. Thus, the kampferol and quercetin 3-diglucosides recently reported<sup>12,13</sup> to occur in the petals of nineteen wild potato species have now been identified as the 3-sophorosides. Also, the 3-di- and 3-triglucoside, 7-rhamnosides of kampferol present in potato seeds yield sophorose on hydrolysis or on oxidation. Further, it is likely on genetic grounds<sup>13</sup> that the 3-glucosylrhamnosyl-glucosides of kampferol and quercetin, occurring in potato flowers with the 3-sophorosides, contain a  $\beta$  1  $\rightarrow$  2 link; the trisaccharide attached to these two pigments may be formulated as glucosyl(1  $\rightarrow$  2)- $\beta$ -rhamnosyl-(1  $\rightarrow$  6)- $\alpha$ -glucose. This formula takes into account the fact

**Summary.** *L*-Leucine is the precursor of the isovaleric acid in magnamycin, as could be demonstrated with *L*-Leucine-[U-<sup>14</sup>].

H. GRISEBACH und H. ACHENBACH

Chemisches Laboratorium der Universität Freiburg i. Br. (Deutschland), 11. September, 1962.

that rutinose, a constituent of the trisaccharide, has recently been found to have an  $\alpha$ - rather than a  $\beta$ -linkage<sup>14</sup>. Again, the quercetin 3-diglucoside 7-glucoside found at the same time<sup>12,13</sup> in another solanaceous plant, the cultivated *Petunia*, is now shown to be the 3-sophoroside 7-glucoside. Independent confirmation of this conclusion is provided by BIRKOFER and KAISER<sup>15</sup>, who have just isolated this pigment, the corresponding kampferol derivative and the two related sophorosides from the same plant. Quercetin 3-sophoroside 7-glucoside also occurs (acylated with caffeic acid) in the flowers of *Helleborus foetidus* (fam. Ranunculaceae).

Kampferol and quercetin 3-sophorosides have also been detected in the leaves of *Pisum* (both wild and cultivated)<sup>16</sup> and in the petals of most wild and garden roses. In the case of *Pisum*, it is clear that the 3-triglucosides, which accompany the 3-sophorosides and which occur in acylated form, contain one, if not two,  $\beta$  1  $\rightarrow$  2 linkages. These acylated triglucosides have been studied in some detail by other workers<sup>17,18</sup> because of their inhibitory effect on indoleacetic acid oxidase. The kampferol and quercetin 'monoglucosides' reported to occur in the variety 'Alaska'<sup>18</sup> must, from their Rf-values and from our own studies, be 3-sophorosides or 3-triglucosides. Another related pigment occurs characteristically in the field pea, *Pisum arvense*; it is an acylated kampferol 3-galactosylsophoroside. In *Rosa*, the 3-sophorosides have been detected in 14 out of 25 species examined; they occur here in association with the corresponding 3- and 4'-glucosides.

<sup>1</sup> J. RABATÉ and J. DUSSY, Bull. Soc. Chim. biol. 20, 467 (1938).<sup>2</sup> E. VIS and H. G. FLETCHER, J. Amer. chem. Soc. 78, 4709 (1956).<sup>3</sup> K. FREUDENBERG, H. KNAUBER, and F. CRAMER, Ber. dtsch. chem. Ges. 84, 144 (1951).<sup>4</sup> M. J. CLANCY, J. chem. Soc. 1960, 4213.<sup>5</sup> W. KARRER, Konstitution und Vorkommen der organischen Pflanzenstoffe (Birkhäuser-Verlag, Basel 1958), p. 264.<sup>6</sup> K. E. GROVE, M. INUBUSE, and R. ROBINSON, J. chem. Soc. 1934, 1608.<sup>7</sup> J. B. HARBORNE and H. S. A. SHERRATT, Biochem. J. 78, 298 (1961).<sup>8</sup> H. H. STROH, Z. Naturf. 14b, 699 (1959).<sup>9</sup> P. KARRER and G. DE MEURON, Helv. chim. Acta 15, 507, 1212 (1932).<sup>10</sup> B. V. CHANDLER and K. A. HARPER, Austral. J. Chem. 14, 586 (1961).<sup>11</sup> J. B. HARBORNE, Phytochemistry, in press.<sup>12</sup> J. B. HARBORNE, Chem. and Ind. 222 (1962).<sup>13</sup> J. B. HARBORNE, Biochem. J. 84, 100 (1962).<sup>14</sup> P. A. J. GORIN and A. S. PERLIN, Canad. J. Chem. 37, 1930 (1959).<sup>15</sup> L. BIRKOFER and C. KAISER, Z. Naturf. 17b, 359 (1962).<sup>16</sup> K. S. DODDS and J. B. HARBORNE, unpublished observations.<sup>17</sup> F. E. MUMFORD, D. H. SMITH, and J. E. CASTLE, Plant Physiol. 36, 752 (1961).<sup>18</sup> M. FURUYA, A. W. GALSTON, and B. B. STOWE, Nature, Lond. 193, 456 (1962).

Jointly, these results show that sophorose, far from being a rare sugar, is the commonest glucose-containing disaccharide associated with the flavonoids. Gentiobiose is the only other glucose disaccharide yet found as a component of flavonoids, and pigments with this sugar are only known to occur in two plants, namely *Primula sinensis*<sup>7</sup> and *Tritonia*<sup>11</sup>.

It is interesting to compare this finding of glucosyl (1 → 2)- $\beta$ -glucose in flavonoid pigments with the situation in plant polysaccharides, in which  $\beta$  1 → 4 and  $\beta$  1 → 6 links are the most common; and with the situation in phenolic glycosides, formed when phenols are fed to plants, in which the only linkage found is  $\beta$  1 → 4<sup>19,20</sup>. The enzyme system catalysing the synthesis of  $\beta$  1 → 2 links thus appears to be rather localized in its distribution in the plant and to display a considerable degree of specificity. This is borne out by the fact that among all the plants so far found to contain sophorose, no example has been found where anthocyanins and flavones co-occurring are both linked to this sugar. This, in effect, means that whenever a second glucose is transferred to a flavonoid monoglucoside, the system catalysing the transfer is specific for anthocyanidin or flavonol. *Primula sinensis*, in which related anthocyanidin and flavonol 3-

gentiobiosides and 3-triglucosides have been found<sup>7</sup>, is the only known exception to this rule<sup>21</sup>.

**Zusammenfassung.** Der seltene Zucker Sophorose konnte mit der Disaccharid-Einheit von Anthozyanen identifiziert werden, die in Arten von *Brassica*, *Papaver*, *Phaseolus*, *Raphanus*, *Tropaeolum* und *Watsonia* vorkommen, und von Flavonen, die in *Helleborus*, *Petunia*, *Pisum*, *Rosa* und *Solanum* vorhanden sind. Es scheint das am häufigsten auftretende, glucosehaltige Disaccharid mit diesen Farbstoffen übereinzustimmen.

J. B. HARBORNE

*John Innes Institute, Bayfordbury (Hertford, Herts, England), August 21, 1962.*

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<sup>21</sup> The author gratefully acknowledges the assistance of Dr. J. B. PRIDHAM in carrying out the electrophoretic experiments.

### The Effect of some Surface Active Drugs on the Assimilation of Ammonium Ions by a Strain of *Pseudomonas aeruginosa*

There is general agreement that surface active agents are adsorbed on the cell surface of bacteria and yeast. At certain concentrations this adsorption results in a leakage from the cell of potassium<sup>1,2</sup>, amino acids<sup>3</sup>, purines and pyrimidines<sup>4</sup> and undoubtedly other cell constituents. At the same time the oxygen uptake and CO<sub>2</sub> production may be increased<sup>5,6</sup> and certain substrates not oxidized by the normal cell may be oxidized presumably because the substrate has access to the enzyme once a barrier has been removed by the drug<sup>1</sup>. Some enzymes are inhibited<sup>7-10</sup>.

In the following, it will be shown that a strain of *Pseudomonas aeruginosa* assimilates ammonium ions in the presence of an oxidizable substrate and sodium and potassium ions. In the absence of potassium ions very little assimilation occurs. Assimilation can be restored however by the addition of a proper concentration of either polymyxin B or benzalkonium chloride. The anionic detergent sodium alkylbenzene sulfonate is

relatively ineffective although it and the cationic compounds may increase the oxidation rate of the substrate.

A strain of *Pseudomonas aeruginosa* kept in this laboratory for fifteen years was grown for 24 h at 34° on Bacto Nutrient Broth. The cells were then centrifuged down, washed twice with water and finally suspended in 0.05 M Na phosphate buffer to a standard optical density. Despite this, there was considerable variation from experiment to experiment. Since the number of molecules

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<sup>2</sup> R. D. HOTCHKISS, Ann. N.Y. Acad. Sci. **46**, 479 (1946).

<sup>3</sup> E. F. GALE and E. S. TAYLOR, J. Gen. Microbiol. **1**, 77 (1947).

<sup>4</sup> M. R. J. SALTON, J. Gen. Microbiol. **5**, 391 (1951).

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<sup>6</sup> I. BIHLER, A. ROTHSTEIN, and L. BIHLER, Biochem. Pharm. **8**, 289 (1961).

<sup>7</sup> M. G. SEVAG and O. A. ROSS, J. Bact. **48**, 677 (1944).

<sup>8</sup> T. G. SCHARF and J. L. BECK, Proc. Soc. exp. Biol. Med. **100**, 307 (1959).

<sup>9</sup> O. RAHN and W. P. VAN ESELTINE, Ann. Rev. Microbiol. **1**, 173 (1947).

<sup>10</sup> W. McD. ARMSTRONG, Arch. Biochem. Biophys. **71**, 137 (1952).

The effect of 10  $\mu$ g/ml polymyxin B, 5  $\mu$ g/ml benzalkonium chloride, 80  $\mu$ g/ml sodium alkylbenzene sulfonate, and 0.025 M potassium phosphate pH 7.7 on the oxidation of 1.0 mg sodium succinate (6H<sub>2</sub>O) on the assimilation of ammonium ions. The cells were suspended in 0.05 M sodium phosphate buffer pH 7.7 except when potassium was present when 0.025 M sodium buffer was used. The concentrations of the drugs are optimal or very near it. 26  $\mu$ g of NH<sub>3</sub>-N was added as NH<sub>4</sub>Cl

Additions	Incubation time min	O <sub>2</sub> uptake $\mu$ l	$\mu$ g NH <sub>3</sub> -N assimilated	$\mu$ g NH <sub>3</sub> -N $\times$ 100 $\mu$ l O <sub>2</sub>	Mean and S.D. of ratio for 6-8 experiments
None	80	113	4	3.5	2.5 $\pm$ 1.8
Benzalkonium chloride	80	107	9	8.4	8.6 $\pm$ 3.4
Polymyxin B	80	129	9	7.0	7.3 $\pm$ 4.1
Sodium alkyl benzene sulfonate	80	137	6	4.4	4.3 $\pm$ 1.3
KH <sub>2</sub> PO <sub>4</sub>	40	85	8	9.4	10.5 $\pm$ 1.4