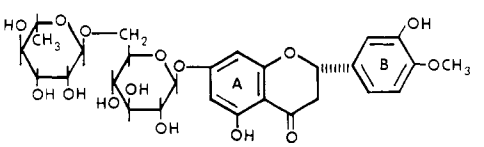
Taste and Structure in Phenolic Glycosides

R. M. Horowitz and Bruno Gentili

Certain intensely bitter flavanone glycosides occur in citrus fruits together with isomeric glycosides that are tasteless. The bitter compounds all contain the disaccharide β-neohesperidose (2-*O*-α-L-rhamnosyl-β-D-glucose). The tasteless compounds contain an isomeric disaccharide, β-rutinose (6-*O*-α-L-rhamnosyl-β-D-glucose). When alterations are made at selected sites in the flavanone neohesperidosides the product may be bitter, bittersweet, sweet, or tasteless. Corresponding changes made in the flavanone rutinosides usually result in tasteless compounds unless the rhamnose is removed from the 6-position of glucose. Of particular interest in this series are the neohesperidosyl dihydrochalcones, several of which are intensely sweet. This paper reviews recent findings in this field.

The peels of oranges, lemons, and grapefruit contain an array of flavonoid compounds of diverse type and structure. Two of the best known of these compounds are hesperidin, the main flavonoid constituent of oranges and lemons, and naringin, the main flavonoid constituent of grapefruit. These substances have been known for more than a century and are characterized by their abundance, accessibility, and ease of isolation. More important, there are a number of interesting taste phenomena associated with these and related compounds, and it is this aspect with which we will be principally concerned.

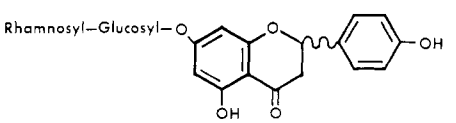
The combined effort of many workers has shown that hesperidin has structure I (*cf.* Horowitz, 1964). It is composed of three parts: L-rhamnose is linked α1 → 6 to D-glucose, which, in turn, is linked β to the C-7 hydroxy



I

group of the flavanone 2(*S*)-hesperetin. 6-*O*-α-L-Rhamnopyranosyl-D-glucopyranose, the disaccharide component of hesperidin, has been given the trivial name rutinose, since it was first found in the widely occurring glycoside, rutin.

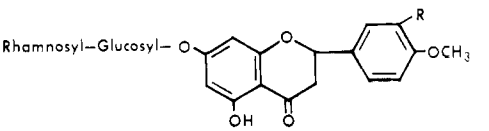
Naringin, too, is a flavanone rhamnosylglucoside and its structure has many features in common with that of hesperidin. Until a few years ago, when we undertook a new study of these compounds, naringin was represented as II. The aglycone portion is the flavanone naringenin,



II

which differs from hesperetin only in the B-ring substitution pattern, and which is shown here with unspecified stereochemistry. The linkage of rhamnose to glucose had not been determined for naringin, but it was assumed by many authorities that it was likely to be 1 → 6, as in hesperidin.

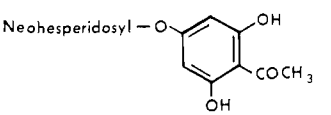
Let us consider two other citrus flavanones that were known at the time: poncirin (from *Poncirus trifoliata*) (Hattori *et al.*, 1944) and neohesperidin (from the Seville orange, *Citrus aurantium*) (Kolle and Gloppe, 1936). These were represented by structures III and IV, respec-



III: R = H
IV: R = OH

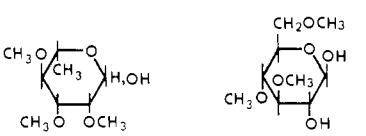
tively. Again, the exact structure of the sugar portion of these substances was not known, although Zemplén and Tettamanti (1938) recognized that the disaccharide in neohesperidin was not identical with rutinose. Neohesperidose was the name they chose for this new disaccharide of still unknown constitution.

Our initial experiment in this field was to treat neohesperidin (IV) with hot alkali and thereby obtain a crystalline degradation product, phloracetophenone 4′-neohesperidoside (V), which was formed from the parent molecule by loss of the B-ring together with the attached carbon atom. It is significant that V was also obtained when either naringin (II) or poncirin (III) was similarly treated. This proved that glycosides II, III, and IV are all derivatives of neohesperidose and immediately the



V

question of the exact structure of the disaccharide reasserted itself. This was answered by three concurring pieces of evidence, of which we will mention only the two most significant. First, since exhaustive methylation of naringin followed by acid hydrolysis yielded the methylated sugars 2,3,4-tri-*O*-methyl-L-rhamnopyranose (VI) and 3,4,6-tri-*O*-methyl-β-D-glucopyranose (VII), it was clear



VI VII

that the rhamnose in neohesperidose is linked 1 → 2 to glucose. Secondly, we were able to confirm this by synthesizing the 1 → 2 linked disaccharide and comparing

Fruit and Vegetable Chemistry Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Pasadena, Calif. 91106

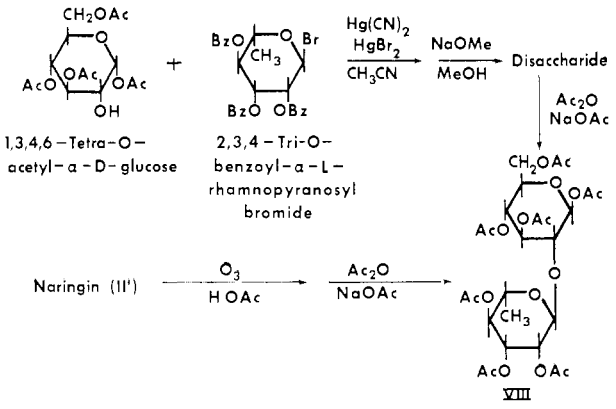
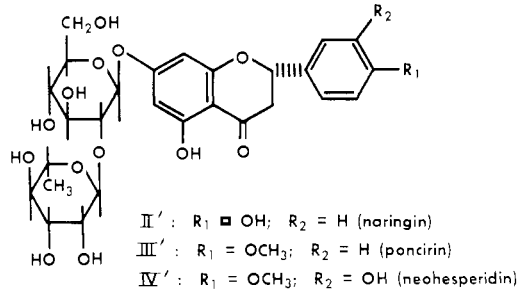


Figure 1. Synthesis of neohesperidose β-heptaacetate (VIII)

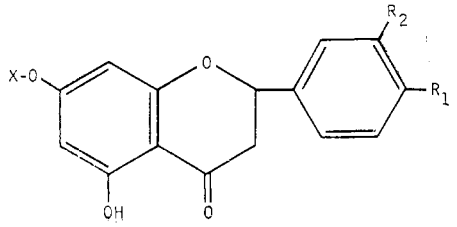
it with a sample of neohesperidose obtained from the natural source, as shown in Figure 1 (Horowitz *et al.*, 1964). The configuration of rhamnose was determined to be α by the method of synthesis, by optical rotational data, and by the fact that virtually all naturally occurring glycosides of L-sugars are α-linked. Neohesperidose is, therefore, 2-*O*-α-L-rhamnopyranosyl-D-glucose and, when it is bound as a glycoside, the glucose moiety has the β-D-glucopyranosyl form. We can now rewrite the structures of naringin, poncirin, and neohesperidin as the β-neohesperidosides II,′ III,′ and IV,′ respectively. The aglycone in these structures has the 2(*S*)-configuration (Gaffield and Waiss, 1968; Gaffield, 1969).



Although these results are of phytochemical interest, it is unlikely that they would have drawn more than routine attention had they not suddenly provided the explanation for an old and very puzzling observation. It had long been known that naringin is an intensely bitter compound (in fact, the main bitter principle in grapefruit), while hesperidin is essentially tasteless. There had not appeared to be sufficient difference in the chemical structures of these two compounds, as understood previously, to account for such a major difference in taste. With the new information on the structure of the disaccharide components of these glycosides, the discrepancy in taste properties appeared to have a much more reasonable structural basis. Moreover, it was gratifying to discover that, in addition to naringin, the other two flavanone β-neohesperidosides, poncirin (III′) and neohesperidin (IV′), were intensely bitter. Thus, to judge from the evidence, it seemed reasonably clear that flavanone 7-β-neohesperidosides were bitter, that flavanone 7-β-rutinosides were tasteless, and that the point of attachment of rhamnose to glucose was the determining factor for bitterness or tastelessness.

At the time this work was done the compounds we have been discussing were the only flavanone glycosides known in citrus. Subsequently, we succeeded in isolating four additional flavanone glycosides and in determining their structures. The compounds known at present are shown in Table I.

Table I. Flavanone Glycosides of Citrus Fruits



X = Rutinosyl	X = Neohesperidosyl	R_1	R_2
Hesperidin	Neohesperidin	OMe	OH
Naringenin rutinoside	Naringin	OH	H
Isosakuranetin rutinoside	Poncirin	OMe	H
Eriocitrin	Neoeriocitrin	OH	OH

Table II. Molar Concentrations of Isobitter Solutions of Flavanone Neohesperidosides and Quinine

Compound	Molarity	Relative Bitterness
Neoeriocitrin	$>5 \times 10^{-4}$	<2
Neohesperidin	5×10^{-4}	2
Naringin	5×10^{-5}	20
Poncirin	5×10^{-5}	20
Quinine dihydrochloride	1×10^{-5}	100

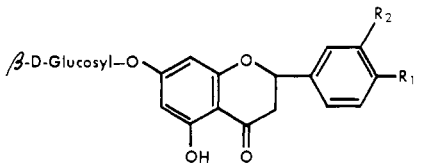
An interesting feature of this table is that of the four different flavanone aglycones shown, each is represented by both a β-rutinosyl and a β-neohesperidosyl derivative. Of more significance, however, is the fact that the taste-structure relations that we had deduced earlier by examining only a relatively few compounds have survived unscathed throughout the series. Thus, all the flavanone rutinosides in Table I are tasteless and all the flavanone neohesperidosides are bitter. As would be expected, there is a good deal of variation in degree of bitterness between members of the series. The relative bitterness of these compounds compared with quinine is shown in Table II. Even though the flavanones are less bitter than quinine, they still must be put in the category of intensely bitter compounds.

Having thus established the relation between structure and bitterness, we decided to explore further in order to map out, if possible, some of the more prominent structural requirements for taste. This work has now branched out considerably and we will attempt here only to summarize the more salient features.

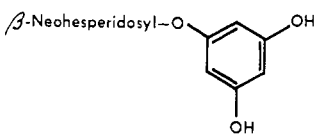
Our general approach has been to make various modifications in the naturally occurring flavanone glycosides. These modifications have taken the following forms: (1) the removal of large fragments of the molecule; (2) the conversion of the flavanone aglycone into other flavonoid types; and (3) the alteration of substituent groups. We find that modifications of types 1 or 3 generally give rise only to quantitative changes in the existing taste, while modifications of type 2 may, in certain cases, give rise to qualitative changes. Examples of each of these kinds of modification will be discussed.

(1) REMOVAL OF LARGE FRAGMENTS

By this we mean loss of one or both sugars, or loss of the B-ring together with one or more carbon atoms of the heterocyclic ring. The effect of losing a sugar is seen, for example, in naringenin 7-β-D-glucoside (prunin) (IX) and hesperetin 7-β-D-glucoside (X), which retain all or some of the bitterness of the parent compounds. The fact that these compounds are bitter at all shows that intact neohesperidose is not required for bitterness. To judge from the available quantitative data it appears that the effect of



IX: R_1 = OH; R_2 = H
X: R_1 = OCH_3; R_2 = OH



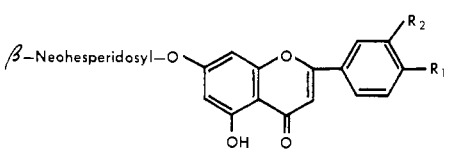
XI

adding an α-L-rhamnosyl residue to the C-2 hydroxyl of D-glucose (to give neohesperidosyl) is to enhance or, at least, maintain bitterness, while the effect of adding it to the C-6 hydroxyl (to give rutinosyl) is to abolish it entirely. When both rhamnose and glucose are lost the solubility of the aglycone is greatly diminished and the taste is usually nil.

The loss of the B-ring under alkaline conditions affords phloracetophenone 4′-β-neohesperidoside (V) or, in certain cases, phloroglucinol β-neohesperidoside (XI). The former compound is intensely bitter; the latter is tasteless. We conclude from these and other data that the carbonyl group is probably required for bitterness.

(2) CONVERSION TO OTHER FLAVONOID TYPES

The oxidation of naringin (II′) and neohesperidin (IV′) to the corresponding flavones, rhoifolin (XII) and neodiosmin (XIII), causes a loss of bitterness and the formation



XII: R_1 = OH; R_2 = H
XIII: R_1 = OCH_3; R_2 = OH

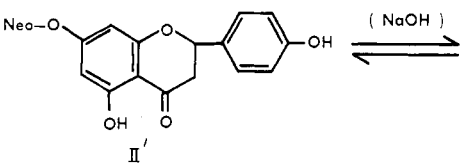
of tasteless compounds. A solution of naringin containing a large amount of rhoifolin is less bitter than naringin alone. This suggests that rhoifolin is able to compete with naringin for sites on the taste receptors, though it does not produce a taste response of its own.

Data of this type led us to infer that highly conjugated, planar aglycones tend to abolish taste responses, while less conjugated, nonplanar aglycones favor them. To confirm the point we prepared the chalcone (XIV) and dihydrochalcone (XV) corresponding to naringin, our expectation being that the planar chalcone would be tasteless and the

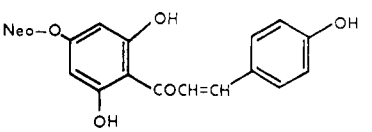
Table III. Taste and Relative Sweetness of Dihydrochalcone Neohesperidosides and Saccharin

Compound	Taste	Molarity of Isosweet Soln.	Relative Sweetness	
			Molar	Weight
Naringin DHC	Sweet	2×10^{-4}	1	0.4
Neohesperidin DHC	Sweet	1×10^{-5}	20	7
Neoeriocitrin DHC	Sl. sweet	...	...	...
Poncirin DHC	Sl. bitter	...	...	...
Saccharin (Na)	Sweet	2×10^{-4}	1	1

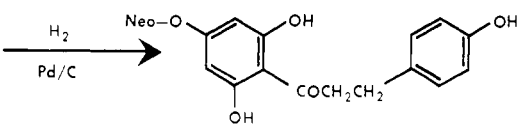
nonplanar dihydrochalcone would be bitter. The reactions involved are as follows.



II′



XIV



XV

In fact, both the chalcone and dihydrochalcone proved to be intensely sweet (Horowitz and Gentili, 1963; Horowitz, 1964). It seems clear that we must devise more subtle explanations than that of mere planarity or nonplanarity to account for these results.

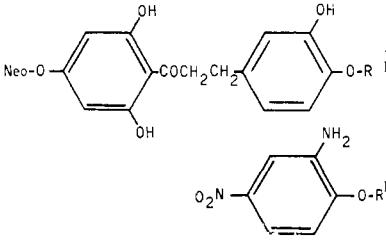
Because of the unexpectedness of this finding, the remaining bitter flavanone neohesperidosides listed in Table I were also converted to their dihydrochalcones. The results for the four compounds are shown in Table III.

Two of the dihydrochalcones, those from naringin and neohesperidin, have exceedingly high levels of sweetness in view of the fact that saccharin, the substance used for comparison, is said to be about 300 times sweeter than sucrose. Poncirin dihydrochalcone is the only nonsweet compound in the group and is actually somewhat bitter. We have found in subsequent work that there must be at least one hydroxyl group in the B-ring for sweetness to subsist in the dihydrochalcones.

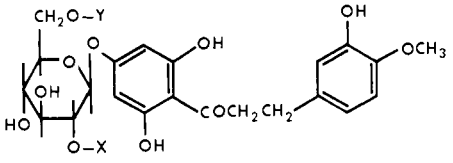
In contrast to the results with flavanone neohesperidosides, flavanone rutinosides such as hesperidin or naringenin 7-β-rutinoside (Table I) yield only tasteless dihydrochalcones. Thus, the influence of the rutinosyl radical in abolishing the taste response is very strong and is manifested in the dihydrochalcones as well as in the flavanones. We can, however, produce a sweet substance from hesperidin dihydrochalcone (XVI) by hydrolyzing rhamnose to give hesperetin dihydrochalcone glucoside (HDG) (XVII). This compound can also be made by partial hydrolysis of neohesperidin dihydrochalcone (XVIII). HDG is about $^1/_{20}$ as sweet as the latter compound and is less soluble.

To recapitulate, most of the taste phenomena we have

Table IV. Relative Sweetness of a Series of Dihydrochalcones and 4-Nitro-2-aminophenyl Ethers



R	
n-Pr > Et ≅ Me > i-Pr	
n-Pr > Et > i-Pr > Me	



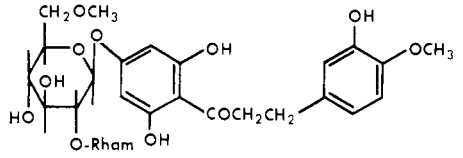
XVI: X = H; Y = α-L-rhamnosyl
XVII: X = Y = H
XVIII: X = α-L-rhamnosyl; Y = H

been discussing are exhibited to some extent by the simple β-D-glucosides of the phenolic aglycones. The attachment of α-L-rhamnosyl to the C-2 hydroxyl of glucose usually enhances the taste and increases the water solubility, while its attachment to the C-6 hydroxyl destroys the taste. We note here that the free disaccharide, neohesperidose, is itself only very slightly sweet, and free rutinose is essentially tasteless.

(3) ALTERATION OF SUBSTITUENT GROUPS

Most of these alterations involve the alkylation of free A- or B-ring phenolic hydroxyl groups. As a rule, methylation or ethylation of one or more of these groups causes a decrease in the bitterness of flavanones, a decrease in the sweetness of dihydrochalcones, and a decrease in solubility of almost every compound. An interesting exception has been uncovered by Krbechek *et al.* (1968), who studied the effect of lengthening the chain of the 4-alkoxy group in neohesperidin dihydrochalcone. Replacement of the 4-methoxy with a 4-ethoxy gave little change, but replacement with a 4-*n*-propoxy gave a twofold increase in sweetness. We have prepared the 4-isopropoxy derivative and find it less sweet than any of the others in the series. The relevant structures and order of sweetness are given in Table IV. It is interesting to compare this series with the corresponding 4-nitro-2-aminophenyl alkyl ethers (Table IV), which are reported to be intensely sweet (Moncrieff, 1967). One is tempted to speculate that the compounds in the two series act on the same set of taste receptors by similar mechanisms.

Instead of altering the phenolic hydroxyl groups one can alter the sugar hydroxyls, though this requires more tedious synthetic procedures. It is conceivable that experiments of this sort will throw light on the challenging question of the difference in the taste properties of the rutinose and neohesperidose substituted compounds. As mentioned earlier, the taste responses produced by glucosides and neohesperidosides are qualitatively similar. A feature shared by these two groups of glycosides is the presence of the free C-6 hydroxyl group in glucose. This is the only primary hydroxyl in the molecules and it is, of course, absent in the rutinosides where it is blocked by rhamnose. It seemed reasonable to suppose that this primary hydroxyl group must be involved in taste stimulation, possibly because it attaches itself to a taste site by hydrogen bonding. We therefore methylated the glucose C-6 hydroxyl in neohesperidin dihydrochalcone to see whether blocking the group would abolish the sweet taste. To our surprise the taste properties of the product, 6″-*O*-methylneohesperidin dihydrochalcone (XIX) were virtually indistinguishable



XIX

from those of the parent compound. From these results we have reached some tentative conclusions about structural specificity in the sugar part of the molecule.

Neither the C-2 nor C-6 hydroxyl of glucose is required for taste, since taste is not abolished by blocking them with a 2-*O*-α-L-rhamnosyl substituent or a 6-*O*-methyl substituent. Furthermore, the C-2 and C-6 hydroxyls can be absent simultaneously without affecting the taste (see XIX).

From this we infer that the structural features most directly involved in taste are the C-3 and C-4 hydroxyl groups of glucose. A similar conclusion was reached by Evans (1963) in studies on taste receptor stimulation in the blowfly. When a series of D-glucose derivatives was tested it appeared that only the C-3 and C-4 hydroxyl groups of glucose were effective in combining with the receptor sites. A further analogy is found in the antibody-antigen like reactions of the plant agglutinins with the blood group substances. These reactions can be inhibited by mono- or oligosaccharides and the extent of inhibition is determined by the stereochemistry of the C-3 and C-4 hydroxyl groups of the terminal sugar of the inhibitor (Boyd, 1962). If there is validity in this view of the importance of the C-3 and C-4 hydroxyl groups one would expect that epimerizing or alkylating one or both of these hydroxyls should have a marked effect on taste responses.

A bulky substituent such as L-rhamnosyl can have opposing effects depending on where it is attached to glucose. When linked at the C-2 position the over-all shape of the molecule is favorable for attachment to the taste receptor site. In addition, the hydroxyl groups of the rhamnose probably enhance binding to the site and consequently the taste. At the C-6 position the over-all shape is unfavorable to such an extent that attachment to the receptors is strongly inhibited and the taste abolished. On the other hand, a C-6 methyl group is small enough that it does not appreciably interfere with binding and consequently the taste of 6″-*O*-methylneohesperidin dihydrochalcone (XIX) is similar to that of the parent compound. If these views are correct we would expect that substituting other pentoses or hexoses for rhamnose would neither seriously impair the taste if attached at C-2 nor produce a taste if attached at C-6.

The discovery that the intensely bitter flavanone neohesperidosides can be converted to intensely sweet dihydrochalcone neohesperidosides conceivably has practical significance. The presently accepted artificial sweeteners, saccharin and cyclamate, have enjoyed a steadily increasing demand during the last decade. Neither they nor the dihydrochalcones are without their own particular set of

flaws. In the case of the dihydrochalcones, although the sweetness is intense and is not marred by a bitter aftertaste, it is rather slow in its onset, is felt mainly in the back part of the mouth, is repetitive, and of long duration. It is described by some as having a slight licorice or menthol-like quality. On the other hand, the dihydrochalcone sweeteners seem to be remarkably free of toxicity in laboratory animals. In experiments carried out by Booth and Robbins (1968), when neohesperidin dihydrochalcone or naringin dihydrochalcone was fed to rats for 170 days at the very high level of 5% of the diet no abnormal pathology, changes in growth rate, or toxic effects of any kind were observed. This is in line with the fact that flavonoids, as a group, are innocuous and that they are metabolized by the intestinal flora to carbon dioxide (from the A-ring) and various aromatic acids (from the B-ring), none of which can be considered harmful (Booth and Williams, 1963; Masri *et al.*, 1959). Another favorable aspect of the dihydrochalcones is the fact that they contain neither nitrogen nor sulfur, elements that are commonly present in toxic compounds.

It is conceivable, then, that the dihydrochalcones will eventually be used in combination with other sweeteners or in special applications requiring long-lasting sweetness. Whether it will be possible to make derivatives of these compounds having a more sugar-like taste, while retaining the nontoxic characteristic, remains to be seen. Much effort is being directed to this end in various laboratories.

LITERATURE CITED

Booth, A. N., Robbins, D. J., Agricultural Research Service Publication CA 74-18, Western Regional Research Laboratory, U. S. Department of Agriculture, Albany, Calif. 94710, 1968.

Booth, A. N., Williams, R. T., *Biochem. J.* **88,** 66P (1963).

Boyd, W. C., "Introduction to Immunochemical Specificity," pp. 72–84, Interscience, New York, 1962.

Evans, D. R., "Olfaction and Taste," Y. Zotterman, Ed., pp. 165–76, Macmillan, New York, 1963.

Gaffield, W., Western Regional Research Laboratory, U. S. Department of Agriculture, unpublished data, 1969.

Gaffield, W., Waiss, A. C., *Chem. Comm.* **1968,** 29.

Hattori, S., Hasegawa, M., Shimokoriyama, M., *Acta Phytochim.* **14,** 1 (1944).

Horowitz, R. M., in "Biochemistry of Phenolic Compounds," Chap. 14, J. B. Harborne, Ed., pp. 545–71, Academic Press, New York, 1964.

Horowitz, R. M., Gentili, B., U. S. Patent **3,087,821** (April 30, 1963).

Horowitz, R. M., Gentili, B., Hand, E. S., Abstracts of Papers, pp. 158–59, IUPAC International Symposium on the Chemistry of Natural Products, Kyoto, Japan, April 1964.

Kolle, F., Gloppe, K., *Pharm. Zentralhalle* **77,** 421 (1936).

Krbechek, L., Inglett, G., Holik, M., Dowling, B., Wagner, R., Riter, R., J. AGR. FOOD CHEM. **16,** 108 (1968).

Masri, S., Booth, A. N., De Eds, F., *Arch. Biochem. Biophys.* **85,** 284 (1959).

Moncrieff, R. W., "The Chemical Senses," pp. 501-2, CRC Press, Cleveland, Ohio, 1967.

Zemplen, G., Tettamanti, A. K., *Ber. Deut. Chem. Ges.* **71B,** 2511 (1938).

Received for review December 9, 1968. Accepted February 19, 1969. Presented in Symposium on Importance of Nonvolatile Compounds in Flavor, Division of Agricultural and Food Chemistry, 156th Meeting, ACS. Atlantic City, N. J., Sept. 1968.