A Molecular Approach to Flavor Synthesis. I. Menthol Esters of Varying Size and Polarity*

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Synopsis

For a series of menthol esters of increasing size and polarity, "mintyness" decreased rapidly as molecular weight increased. For molecular weights above about 700, the compounds were tasteless to a significant proportion of the taste panel. As polarity increased, the "sweet minty" taste of hydrophobic menthol esters became increasingly bitter, until water-soluble menthol esters were strongly bitter. Bitterness was still apparent in quite high-molecular-weight water-soluble esters; a different receptor may be involved for bitterness than for mint. An overall hypothesis relating flavor to molecular solubility parameters is proposed.

INTRODUCTION

The attachment of flavor groups to polymers is an attractive way to achieve lower volatility, increase flavor retention in the mouth, and avoid absorption of the flavor through the wall of the gastrointestinal tract. In 1975, Zaffaroni of Dynapol[1] obtained a U.S. patent claiming the discovery of nonabsorbable, non-nutritive sweeteners. This patent gives examples of various sweet moieties bound to polymers.

This approach is based on an unstated model which we believe might best be described as a "plugged-in" enzyme model. That is, the taste receptor is enzymelike and somehow electrically connected to the central nervous system. The enzymelike receptor is viewed as having the free access of a soluble species, unencumbered by cellular membranes or any other part of a real cell. The taste buds of mammals have been extensively studied[2] and it appears unlikely that the taste receptor is unencumbered. Kurihara[3] examined the interaction of taste stimuli with a monolayer of lipids extracted from bovine taste papillae. He proposed that salty and sour tastes are induced by interaction of alkali metal ions and protons with the polar groups of phospholipids in the gustatory cell membrane. Bitter taste was believed to arise by penetration of bitter compounds into a nonpolar region of the lipid layer of the membrane. This idea that taste is associated with polarity and/or solubility was also implied by Deutsch and Hansch.[4] Using the relative sweetness data of a series of 2-amino-4-nitrobenzenes, they found a good correlation between sweetness in this series and a "hydrophobic binding constant." This constant is modeled by measuring the partition coefficient of the compound between octanol and water. Therefore, it appears that the "plugged-in" enzyme model must be modified by some sort of overall molecular polarity to allow for diffusion of the molecule into or through

* Dedicated to Professor C. G. Overberger on the occasion of his 60th birthday.

Journal of Polymer Science: Polymer Chemistry Edition, Vol. 18, 2933–2940 (1980)

0360-6376/80/0018-2933$01.00

a lipid membrane. In addition, if taste has an enzymelike binding site under, or associated with, this membrane, we can expect steric problems in binding.[5] Finally, if taste is associated with the binding of a molecule to a receptor, it seems unlikely that the spacing of taste groups on a polymer would be matched by the spacing of receptors. Thus, only one or so groups per polymer could bind, and taste intensity would be low.

We have investigated the taste of menthol esters chemically bound to various hydroxyl-containing polymers and oligomers with the objective of quantifying these qualitative approaches.

Menthol acetate is a standard flavoring agent[6] having a cool feeling in the mouth with a mint flavor. The initial approach was to react menthol and succinic anhydride to give the monomenthyl ester, convert this to the mono acid chloride, and allow this to react with poly(vinyl alcohol), carboxymethylcellulose (CMC), or hydroxypropyl cellulose (Klucel) in dimethylacetamide solvent. The products were tasteless even though a large amount of menthol could be shown to be chemically bound. Based on the hypothesis that this lack of taste was due either to steric hindrance at the menthol binding sites or to molecular size, we prepared esters in which menthol was bound only at the ends of the molecules. The central positions of these molecules were either dibasic acids, polyethylene glycols, or an aliphatic diol. We expected constant steric problems in these series of varying molecular weights.

RESULTS AND DISCUSSION

In order to establish the effect of molecular weight on the taste of menthol esters, the following compounds were synthesized (Table I).

These compounds were tasted as solutions in an odorless, tasteless mineral oil. Solutions were prepared to contain an amount of menthol ester groups equivalent to a 5% menthol acetate standard.

Menthyl acetate was characterized as cool or minty in flavor. The flavor appeared fairly early, peaked at 15–75 sec, and disappeared by 240 sec. Dimenthyl succinate was odorless and tasteless, presumably due to steric inhibition of binding of one menthyl group by the other. The glutarate, adipate, and C_{12} diol derivative (compound 12) were similar to the acetate.

The ethylene glycol derivative (compound 5) did not develop a minty flavor for the majority of the panelists until 15–60 sec. Two panelists out of nine found this sample to be immediately bitter, then minty. The minty flavor peaked later than the acetate at 60–150 sec and lasted longer. Bitterness increased in going from the ethylene glycol to tetraethylene glycol adducts. Going to the polyethylene glycol of degree of polymerization (DP) of 90 (compound 9), bitterness became so strong and vile that one panelist threatened to quit. The most obvious change in this series of ethylene glycol oligomers was that they became increasingly soluble in water and decreasingly soluble in mineral oil. Compound 9 was soluble in water at 5% concentration while the other compounds in the series were soluble only below 0.1%.

Under the assumption that this aqueous solubility was associated with bitterness, the sodium salt of monomenthyl succinic acid was prepared. This water-soluble menthol compound was also quite vile and bitter. That it was the menthyl group that was being tasted as bitter in the water-soluble cases was

TABLE I
Menthol Esters

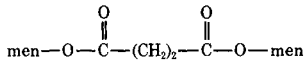
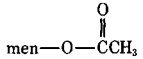
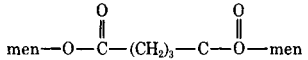
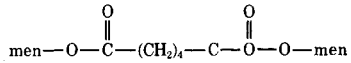
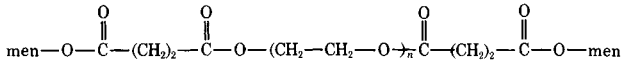
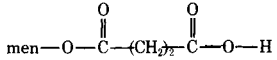
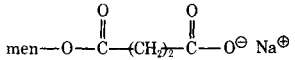
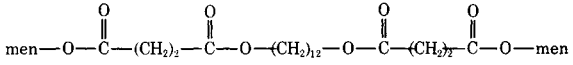
Compound No.	Structure	Name
Group 1: Aliphatic esters		
1		Dimenthyl succinate
2		Menthyl acetate (commercial)
3		Dimenthyl glutarate
4		Dimenthyl adipate
Group 2: Polyethylene glycol derivatives		
5–9	 n = 1, 2, 3, 4, and approx. 90, respectively	
Group 3: Menthol acids		
10		Monomenthyl succinic acid
11		Monomenthyl sodium succinate
Group 4: Aliphatic diol derivative		
12	 men = L-menthol	

TABLE II
Taste Panel Data for Menthol Esters

Compound number	MW	% of panel reporting taste	Peak intensity	Qualitative reports
2	198	100	15–60 sec	Minty
5	538	100	60–150 sec	23% (2/9) reported some bitterness
6–8	670	67	—	Mint taste reported as weak by tasters
9	~3000	100	Rapid	Intensely vile bitterness
12	678	67	—	Weaker than 8

[a] Molecular weight.

substantiated by the synthesis of the cyclohexyl analogs of the ethylene glycol and polyethylene glycol compounds (compounds 5 and 9) which were neither bitter nor minty.

With respect to the perceived intensity of mintyness, compound 5—the ethylene glycol derivative—was slow in developing mintyness but had a "normal" maximum intensity. Compounds 6, 7, and 8 were found to have no taste by one-third of the panel; the remaining members found the taste to be quite weak. Compound 12, which is roughly the "size" of the tetraethylene glycol adduct but is more lipophilic, had the same low taste level as compound 8. This surprisingly sharp loss of mint taste over a relatively small size difference (see Fig. 1) would seem to be inconsistent with diffusion through a homogeneous membrane or with changes in vapor pressure. Diffusion may be through "channels or tubes" whose dimensions are fairly small. These "channels" seem to exist for mintyness but there is no evidence for their existence in bitterness.

If the taste receptor has any resemblance to an enzyme, then the menthyl group on compounds 5–9 should all be available for binding to an active binding site. The fact that the taste varies so strongly among these compounds implies molecular parameters such as solubility and "size" are of considerable importance. Hence, these features need to be considered together with atomic geometry in any general theory of taste. We, therefore, propose the following hypothesis: Taste receptors are surrounded by media of different polarity varying from oil-like to waterlike. On introduction of a compound into the mouth, this compound will distribute itself among the different media according to its relative solubility or partition constants, and thereby reach these receptors in different concentrations if the molecules are small enough to diffuse in. The molecules can then bind, depending on their binding constants, to the different receptors and presumably generate a signal if their binding constants are high enough. The same taste moiety can generate signals which are interpreted differently by the brain depending on which receptor is activated. In this case, an oil-soluble menthyl ester will reach a lipophilic receptor and generate a "minty" signal, while a water-soluble menthol compound will reach a more hydrophilic receptor and generate a "bitter" signal. Presumably, as the hydrophilic–lipophilic balance is changed in menthol compounds, we can generate a variety of "sweet-minty" to "bitter-minty" flavors. This hypothesis has been further developed and will be reported in a separate article.

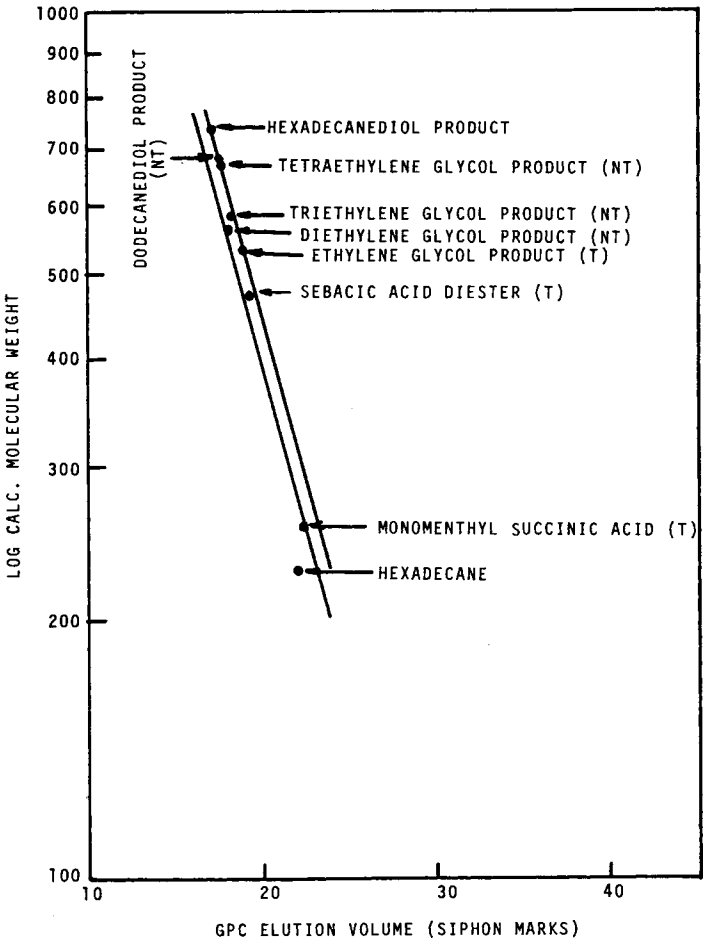


Fig. 1. Monomenthyl succinic acid derivatives: GPC sizes and menthol esters GPC sizes.

EXPERIMENTAL

Monomenthyl Succinate

This compound was first prepared by Arth[7] by reacting succinic acid and menthol, mp ca. 62°C. Reference (8) describes the reaction of succinic anhydride and menthol.

We allowed equimolar quantities of L-menthol (Aldrich Chemical Co.) and succinic anhydride to react in a pop bottle at 100°C until a clear homogeneous liquid was obtained. This required approximately 24 hr; on cooling, the material crystallized. It was recrystallized from hexane to a sharp melting point of 61.5–62.5°C. Crystallization from hexane was very slow.

Monomenthyl Succinoyl Chloride

The procedure of Hancock and Linstead[9] for the conversion of monomethyl succinate to its acid chloride was used. In this procedure oxalyl chloride was used and gave a consistently light-colored product; the use of thionyl chloride

led to darker-colored material. The product, a liquid, showed ester carbonyl at 1745 cm^{-1} and acid chloride at 1800 cm^{-1}.

Esterification of Monomenthyl Succinoyl Chloride

Two procedures were used. The reaction was carried out either in dry dimethylacetamide or in ether-containing triethylamine. The dimethylacetamide procedure was preferred as lighter-colored products were obtained. Dimethylacetamide (DuPont) was first distilled from P_2O_5. The center cut was further dried by passing it through a column containing a top layer of Linde 4-Å molecular sieves and a bottom layer of silica gel. Karl Fisher analyses showed water levels consistently below 1% with most samples having less than 0.5% water.

Reaction was carried out by preparing a solution of the alcohol reactant in this dry dimethylacetamide in a pop bottle, injecting an excess of fresh monomenthyl succinoyl chloride, and allowing the solution to stand overnight. The following morning, crystals presumably of dimethylacetamide hydrochloride, were generally present. The solution was poured into water and the pH increased to 9–10 by adding saturated aqueous sodium carbonate. The water was extracted with chloroform and the chloroform extract was washed with water until the pH of the wash decreased to less than 7. The chloroform extract was then dried over magnesium sulfate and stripped.

If free acid carbonyl was seen in the infrared (IR) (absorption from 1650 to 1725 cm^{-1}), the alkali wash was repeated.

Specific synthesis and analytical data for compounds used are given below. Purity determination was somewhat difficult because most of these esters were nonvolatile oils. In addition, specific analyses were carried out by gas chromatography for free menthol and monomenthyl succinic acid. Menthol was determined at 180°C on a 13-ft × 1/8-in. stainless-steel column packed with Carbowax 20M on Chromosorb W. Monomenthyl succinic acid was determined as the trimethylsilyl derivative. Both materials were less than 1% in all samples and only traces of other compounds were visible.

Reaction Product of Ethylene Glycol and Monomenthyl Succinoyl Chloride (Compound 5)

To a solution of 2.4649 g (0.0398 mole) of dry ethylene glycol in 8 ml of dry dimethylacetamide was added 34.1243 g (0.1241 mole) of monomenthyl succinoyl chloride. After removal of excess monomenthyl succinic acid, 6.39 g (30% yield) of product was recovered.

Gel permeation chromatography (GPC) on a 16 ft × 5/16 in. i.d. Porogel 60-Å column 37-μ particle size showed a single peak at the expected elution volume in tetrahydrofuran (see Fig. 1).

Reaction Product of Tetraethylene Glycol and Monomenthyl Succinoyl Chloride (Compound 8)

To a solution of 10.7154 g (0.0552 mole) of dry tetraethylene glycol dissolved in 40 ml of dry dimethylacetamide was added 48.7453 g (0.1773 mole) of monomenthyl succinoyl chloride. After two procedures to remove excess mo-

nomenthyl succinic acid, 11.91 g (32% yield) of an oil was recovered. Gel permeation chromatography on a 16-ft Porogel 60-Å column showed a single band at the expected elution volume (see Fig. 1).

The Reaction Product of Carbowax 4000 and Menthyl Succinoyl Chloride (Compound 9)

Carbowax 4000 (50 g) (Union Carbide) was dissolved in 55 ml of warm, dry dimethylacetamide and 13.5 g of monomenthyl succinoyl chloride was added. After standing overnight, the contents were poured into 1 liter of pentane, which was decanted, and the solid was washed two more times with pentane, filtered, and dried. The solid was dissolved in 450 ml of warm dioxane and precipitated by dropping this solution into 2 liter of pentane. After drying, the white solid weighed 60.57 g. It was dissolved in 400 ml of 1:1 benzene:dioxane, filtered, and precipitated into 2 liter of rapidly stirred hexane, filtered, and dried; it weighed 51.05 g. The number-average molecular weight, determined in benzene by Mechrolab vapor osmometer, was 2561 ± 147. GPC showed one peak at the void volume of the column.

A second preparation was carried out using 10 g of Carbowax 4000, 40 ml of dimethylacetamide, and 3.4 g of menthyl succinoyl chloride. The reaction mixture was precipitated into ether, washed three times with fresh ether, dissolved in chloroform, and precipitated in ether; yield, 8.68 g.

Monocyclohexyl Succinic Acid

This compound was prepared from 100 g (1 mole) of cyclohexanol (Aldrich) and 100 g (1 mole) of succinic anhydride by the same procedure described for the menthyl ester.

Reaction of Carbowax 4000 and Monocyclohexyl Succinoyl Chloride

Cyclohexyl succinic acid was converted to the acid chloride by the procedure of Hancock and Linstead.[9] Carbowax 4000 (10 g) (Union Carbide) was allowed to react with 2.4 g of cyclohexyl succinoyl chloride in 40 ml of dry dimethylacetamide. The product was worked up by precipitation in ether as described for the menthyl derivative; yield, 9.30 g. The number-average molecular weight was 3020 ± 99 as determined in benzene by vapor osmometry. A single peak was seen in the GPC at the exclusion volume.

Dimenthyl Succinate (Compound 1)

Dimenthyl succinate was prepared from menthol and monomenthyl succinoyl chloride, mp 62–63°C (lit.[7] 62°C).

Dimenthyl Adipate

Prepared from adipoyl chloride and menthol, mp 59.5–60.5°C (lit.[11] 62.5–63.5°C).

Reaction Product of Dodecanediol and Monomenthyl Succinoyl Chloride (Compound 8A)

Prepared from monomenthyl succinoyl chloride and dodecanediol; one peak on GPC (Fig. 1).

The authors wish to thank the staff of the Monell Chemical Senses Center of the University of Pennsylvania for their aid and encouragement, particularly Dr. R. Doty, Dr. C. Christensen, and Dr. M. Kare. A. J. H. would like to thank Hercules for the financial and scientific support to carry out this and other structure activity studies in flavor chemistry.

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Received July 26, 1979
Accepted December 31, 1979