Structural Functions of the Sweet Pharmacophore

G. G. BIRCH *, S. Z. DZIEDZIC *§x, R. S. SHALLENBERGER ‡, and M. G. LINDLEY ‡¶

Received February 26, 1980, from the **National College of Food Technology, University of Reading, Weybridge, Surrey KT13 ODE, England,* and the ‡*New York State Agriculture Experiment Station, Cornell University, Geneva, NY 14456.* Accepted for publication August 18, 1980. §Present address: Department of Food Science, University of Reading, Reading, RG1 5AQ, Berks., England. ¶Present address: Tate and Lyle Ltd., Philip Lyle Memorial Research Laboratory, Reading, RG6 2XB, Berks., England.

Abstract □ The relative sweetness, onset times, and durations of response of D-glucose, D-xylose, D-quinovose, D-galactose, L-arabinose, and D-fucose were determined at four temperatures. The results can be interpreted by simple concepts of intramolecular hydrogen bonding which indicate that the so-called γ-function of the tripartite AH,B,γ sweet pharmacophore plays little or no part in sugar sweetness. Probably the Lemieux effect (intramolecular hydrogen bonding between the hydroxymethyl substituent and the 4-hydroxy group) is of overriding importance in determining sugar sweetness, and the separate features of intensity and time of response indicate distinct functions of chemoreception. The absence of a γ-function in simple hydrophilic molecules such as glucose has already been emphasized. This function distinguishes them from the artificial sweeteners such as saccharin, which may be 500 times or more sweeter than sucrose, depending on their stereostructure and lipophilicity.

Keyphrases □ Sweet pharmacophore—structural function, relative sweetness of six sugars tested □ Sugars—relative sweetness, onset time, and duration of response determined for six sugars, structural functions of the sweet pharmacophore □ Structure–activity relationships—structural functions of six sugars evaluated for sweetness

The AH,B,γ concept of the sweet pharmacophore (1, 2) has been invoked recently (3, 4) to explain differences in sweetness elicited by structurally related molecules. The pharmacophore may be considered (5) as a scalene triangle with the acid function (AH) and the basic function (B) at two of the apexes while the lipophilic function (γ) is at the third apex. The γ-function may be important for sweetness intensity in molecules, such as the aminonitrobenzenes, that have a highly lipophilic character. Its role in sugar molecules is likely to be minimal.

BACKGROUND

Stepwise chemical modification of glucopyranoside molecules (3, 4, 6) indicated that the 3,4-α-glycol is probably the primary AH,B unit. Therefore, the only likely location of the γ-function is the methylene group at C-6 of hexopyranosides.

The galactopyranosides differ from the glucopyranosides only in the configuration of the hydroxyl group at C-4, and their primary AH,B units (O–O interorbital spacings of 2.86 Å) therefore are intrinsically equivalent to the glucopyranosides. However, in galactopyranosides, the hydroxyl group at C-4 forms an intramolecular bond with the ring oxygen and thus is less available than the hydroxyl at C-4 of the glucopyranosides to bind with the taste receptor. Galacto-type structures are generally less sweet than gluco types, although the interplay of hydrogen bonding that governs the sweetness is temperature dependent.

Another type of hydrogen bond that underlies the sweetness of sugars is the intramolecular hydrogen bond between the hydroxymethylene group of glucose (and galactose) and the C-4 hydroxyl. This enhances (5) the acidity of the C-4 hydroxyl proton and has been termed the Lemieux effect (7). Whether such an effect elevates or depresses sweetness in a molecule depends on the molecular structure and temperature. Hydrogen bonds may rupture as the temperature is increased so that effects ascribable solely to their interplay diminish.

Kier (2) and Holtje and Kier (8) explained the contribution of the third dispersion binding moiety to sweet taste and underlined its importance in molecules such as saccharin, neohesperidin dihydrochalcone, and nitroanilines, which are 500–3000 times sweeter than sucrose. Since the low order of sweetness in sugars probably is due to the absence of an effective γ-function, it is of structural interest to modify the γ-effect. Recently, the temporal factor in the sweet response has excited some interest (9, 10), and the persistence of the response has been attributed to an ordered localized concentration of stimulus molecules at or near the receptor. Such a local concentration of molecules may be attributable to the γ-function of the sweet pharmacophore, because the persistence of most simple sugars is similar. Alteration of the γ-function of sugar molecules (*e.g.*, by removal of the oxygen of the hydroxymethyl) would increase lipophilicity and might increase sweetness and persistence. On the other hand, removal of the hydroxymethyl would eliminate the Lemieux effect, in which case the sweetness would diminish.

To explore these fine differences in analogous sugar structures, the sweetness and persistence of six structural analogs in the gluco and galacto series of sugars were investigated. Relative sweetness and persistence values were determined at four temperatures to elucidate the importance of hydrogen bonding in the sweet response.

EXPERIMENTAL

6-Deoxy-α-D-methylglucoside (methyl α-D-quinovopyranoside, I) and D-quinovose (6-deoxy-α-D-glucose, II) were prepared by a series of well-documented procedures in carbohydrate chemistry. D-Glucose (III), D-xylose (IV), D-galactose (V), L-arabinose (VI), D-fucose (VII) α-D-methylglucoside (methyl α-D-glucopyranoside, VIII), and α-D-methylxyloside (methyl α-D-xylopyranoside, IX) were purchased[1] commercially. All compounds were purified by repeated crystallizations before use.

The assessors were selected and trained according to well-established procedures (11). This training involved recognition of suprathreshold levels of the four basic tastes and ranking of different sucrose concentrations.

Equimolar (0.5 *M*) solutions were used, prepared 15 hr prior to tasting so that mutarotational effects were similar. The order of temperatures and presentation was randomized.

Intensity Determinations—Ten panelists were presented with 3-ml samples in sealed glass vials equilibrated to the temperature of tasting. Prior to assessment, panelists were required to rinse their mouths continuously for 20 sec with rinse water provided at that temperature. A method of category scaling (0–10) was used. Panelists were presented with an internal standard (5) and were asked to score the sweetness of the other two solutions accordingly. Two-digit random numbers were used. Tastings were duplicated, *i.e.*, 20 judgments for each compound at each temperature. Continual reference could be made to the standard, provided the mouth was rinsed between tastings. Significance was determined by the Student *t* test.

Temporal Determinations—Immediately before tasting, 12 panelists were asked to rinse their mouths continuously for 20 sec at the specific temperature under examination. At any sitting, three randomly chosen solutions were administered; 50 μl[2] was placed onto the tip of the extended tongue. Panelists communicated visually the onset and persistence of the sweet taste, which were recorded on a stopwatch. Panelists waited at least 2 min before taking the next sample. The results were calculated as an analysis of variance, 4 × 6 × 12 "2 off" unreplicated experiment (*i.e.*, four temperatures, six sugars, 12 panelists). The 95% confidence lists are indicated in all figures by two times the standard error of the mean.

RESULTS AND DISCUSSION

Figure 1 shows the three galacto analogs and the sweetness of L-arab-

[1] Sigma Chemical Co. Poole, Dorset, England.

[2] Oxford dispenser.

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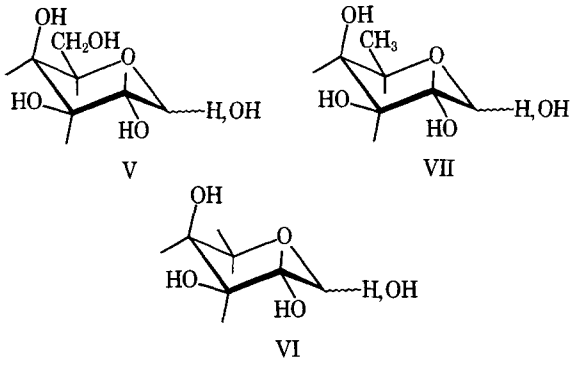


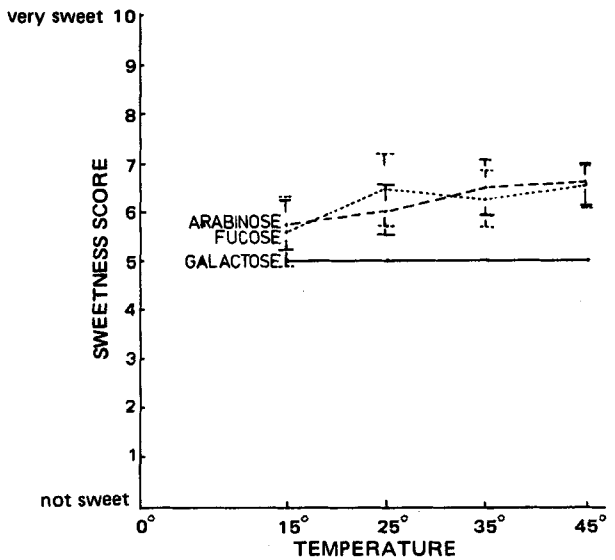
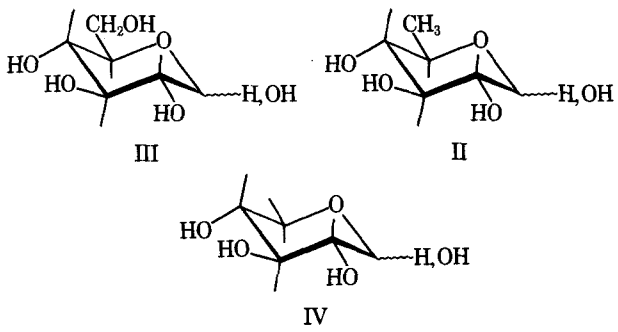


Figure 1—*Sweetness of D-fucose and L-arabinose compared to D-galactose (5) at 15, 25, 35, and 45°. Vertical bars represent the standard error of the mean.*



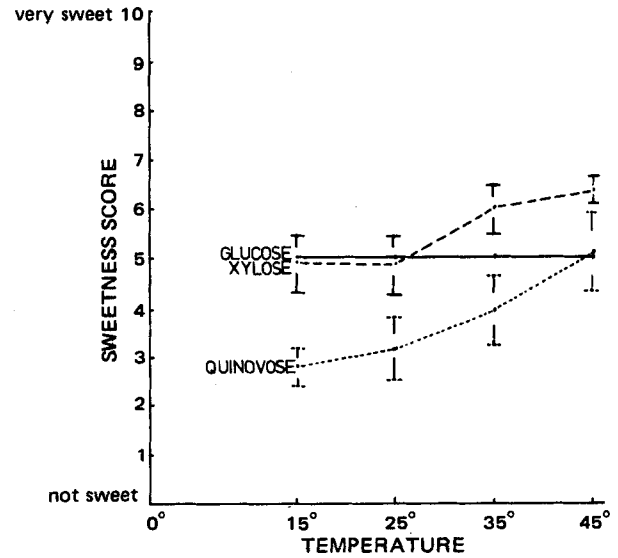


Figure 2—*Sweetness of D-quinovose and D-xylose compared to D-glucose (5) at 15, 25, 35, and 45°. Vertical bars represent the standard error of the mean.*

inose and D-fucose compared to D-galactose (which was given the value 5.0 at each of the four temperatures, 15, 25, 35, and 45°). Both L-arabinose and D-fucose were sweeter than D-galactose throughout this temperature range; this finding is attributable to the two intramolecular hydrogen bonds in the latter sugar. The relative sweetness of L-arabinose and D-fucose increased with increasing temperature because more energy was needed to rupture the doubly intramolecularly hydrogen-bonded galactose molecule. Although D-fucose possesses a γ-function and L-arabinose does not, there was no significant difference in the sweetness of these two sugars throughout the temperature range. This finding illustrates the lack of importance of the γ-site in these analogous structures and emphasizes the overriding property of intramolecular hydrogen bonding and the Lemieux effect, which depresses galactose sweetness.

Figure 2 shows the three gluco analogs and the sweetness of D-xylose and D-quinovose compared to D-glucose over the same temperature range. D-Xylose had a similar sweetness to D-glucose at the lower temperatures, but xylose became significantly sweeter at higher temperatures. Both molecules have the same AH,B system at the 3,4-α-glycol group, but presumably D-xylose, being the smaller molecule, is more sterically favored by the receptor at the higher temperatures. At the lower temperatures, D-glucose undergoes the Lemieux effect, which enhances the acidity of the 4-hydroxy proton and, hence, increases sweetness so that it becomes similar to that of xylose. D-Quinovose has no Lemieux effect (being devoid of a C-4 oxygen) and was significantly less sweet than glucose at lower temperatures. Only at higher temperatures, when the Lemieux effect is no longer operational due to rupture of all intramolecular hydrogen bonds, did D-glucose and D-quinovose become sterically and sensorically equivalent. The γ-site exerted no noticeable effect in any of these results. If it had, D-quinovose would have been sweeter than D-glucose at the higher temperatures.

In a third experiment, the α-D-methylglycosides of the three gluco analogs were tested over the same temperature range. The resulting curves (Fig. 3) were similar to those of the free sugars (Fig. 2); this finding underlines the lack of involvement of the anomeric center.

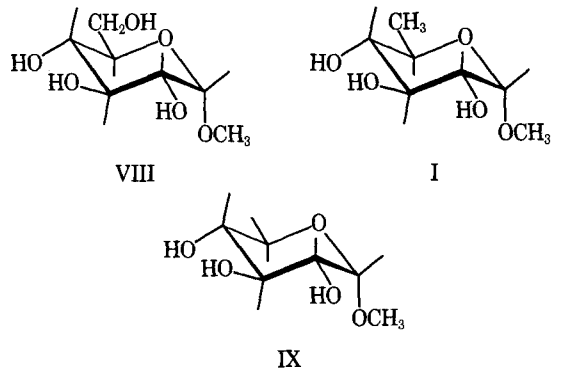
Shallenberger and Lindley (5) reported the dimensions of the scalene triangle (Fig. 4), which specifiy the tripartite sweet pharmacophore. In the gluco and galacto analogs studied, the favored conformation of all six analogs confines the γ-site to C-6 of the hexoses. However, 6-deoxyglucose (quinovose) was less sweet than glucose or xylose at all but the highest temperature studied (Fig. 2), and the same exact pattern was repeated in the glycosides (Fig. 3). Only with 6-deoxygalactose (fucose) was there any increase of sweetness compared with the parent hexose. However, this result can be explained by the doubly intramolecularly hydrogen-bonded galactose structure (Fig. 1) as discussed earlier. Furthermore, fucose had the same sweetness as arabinose, in which the γ-site is absent. Thus, the γ-site is not of major importance in the relative sweetness of these analogous structures.

It was pointed out recently (10) that the temporal factors in sweetness (*i.e.*, onset time and duration of response) may be related to the approach, alignment, and localized concentration of the stimulus molecules in chemoreception. Sugars differ from many artificial sweeteners in that their durations decrease with increasing temperature, and this behavior may be a result of their ineffective γ-sites. If the approach of a stimulus molecule to a sweet receptor governs the onset time and the localized concentration governs the duration of response, it is possible that both may be affected by changes in temperature.

Figure 5 shows that the mean onset time of response of all six sugars decreased with increasing temperature, while Fig. 6 shows that their mean duration likewise decreased. These results are both significant ($p < 0.001$) and agree with those of Larson-Powers and Pangborn (12) for sucrose.

The onset time may be a simple function of temperature-dependent diffusion whereas the duration may represent the accession of sapid molecules to localized concentrations or stores. Thus, the negative temperature effect again reflects the absence of an effective γ-function. The present results also indicate that quinovose had a longer duration of sweetness than the other sugars at all temperatures tested, but there were no other obvious structural differences between the sugars that could have affected their temporal response.

If the γ-site of the sweet pharmacophore is viewed as a function that



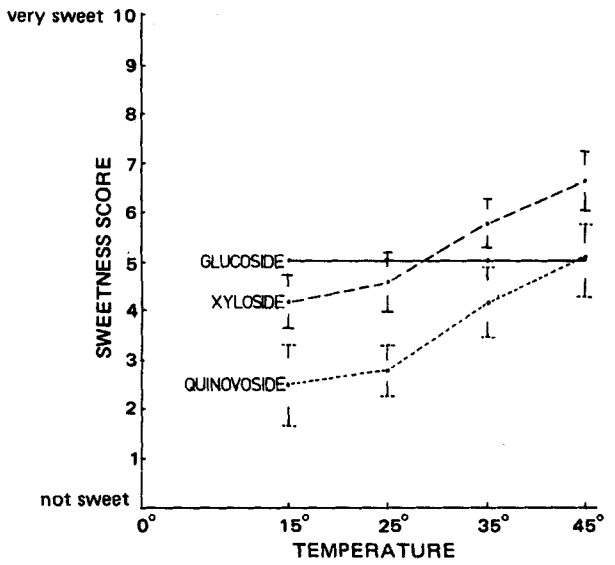


Figure 3—*Sweetness of methyl α-D-xylopyranoside and methyl α-D-quinopyranoside compared to methyl α-D-glucopyranoside at 15, 25, 35, and 45°. Vertical bars represent the standard error of the mean.*

facilitates the accession of stimulus molecules to receptors, 6-deoxyhexoses might show high sweetness intensities and long durations. However, there is no reason to believe that a long duration of response (high localized concentration) should demand a high intensity (occupation of receptors); a more accurate concept of taste chemoreception may involve the distinction of these factors as separate mechanisms (9, 10). Therefore, the observation that quinovose has a longer duration but a lower intensity of sweetness than glucose could be due to its enhanced

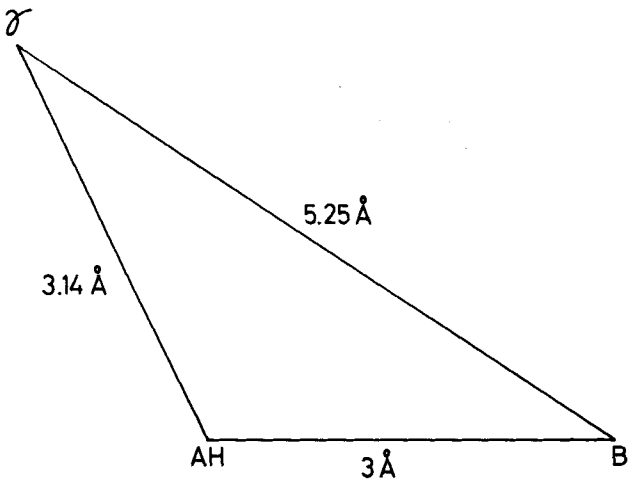


Figure 4—*Dimensions of tripartite sweet pharmacophore represented by scalene triangle (5).*

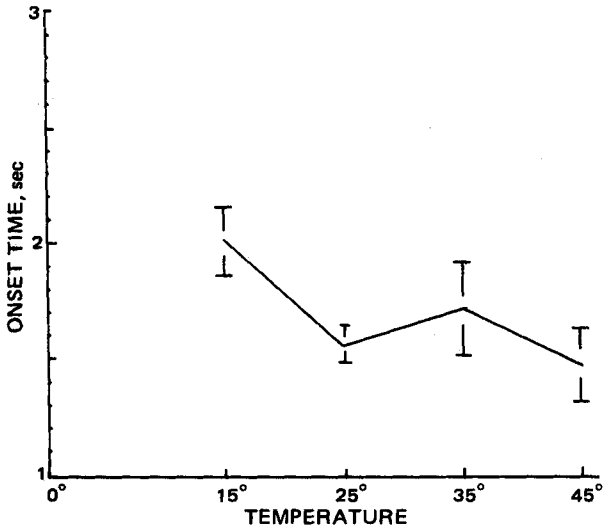


Figure 5—*Mean onset time of sweetness of six analogs at 15, 25, 35, and 45°. Vertical bars represent the standard error of the mean.*

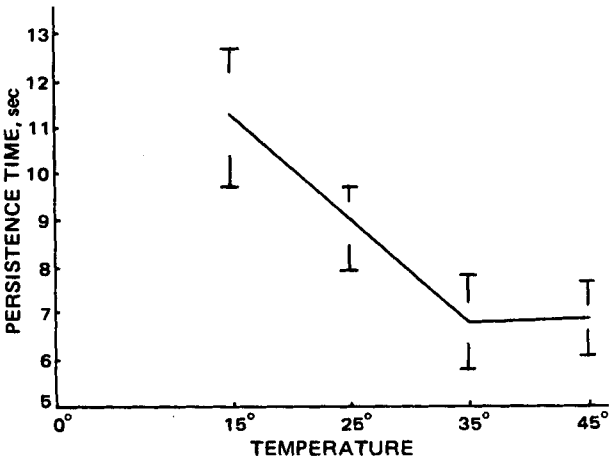


Figure 6—*Mean persistence time (i.e., duration) of sweetness of six analogs at 15, 25, 35, and 45°. Vertical bars represent the standard error of the mean.*

localized concentration at or near the same number of receptors as glucose.

CONCLUSION

Studies of a conformationally and configurationally defined class of sweeteners such as the sugars can result in particular deductions about the sweet pharmacophore. The results reported here underline the dominant role of hydrogen bonding in the sweetness of sugars and offer an explanation of the relative effects of analogous structures. Both intensity and time factors in the sweetness response depend on temperature and may contribute jointly to an understanding of the chemoreception of taste.

Although Kier's (2) γ-function appears to be virtually absent in normal sugar structures, it may be enhanced in 6-deoxyglucose. A more refined and detailed function of the γ-site is postulated in that the latter may govern the approach, alignment, and localized concentrations of sweetener molecules at or near the receptor. These results confirm Kier's (2) original stipulations about the third binding site and extend the concept of the sweet pharmacophore to include intensity–time interactions of the sweet response.

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Crystal Structure and Solid-State Behavior of Aspirin Anhydride Crystals

STEPHEN R. BYRN ˣ **and P. Y. SIEW**

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Abstract □ The crystal structure and solid-state behavior of aspirin anhydride were determined using single-crystal X-ray techniques and microscopic examination of the reacting crystals. The crystal structure and solid-state conformation of aspirin anhydride were similar to those of related compounds. The crystal packing of aspirin anhydride allows the initial product of the solid-state reaction to be predicted; however, this prediction could not be tested because the thermal degradation products reported in the literature appear to be those obtained from reaction in a liquid state.

Keyphrases □ Aspirin anhydride—determination of crystal structure and solid-state behavior, X-ray crystallography and microscopy of reacting crystals □ Crystal structure—aspirin anhydride, determination of solid-state behavior of crystals □ Degradation, thermal—aspirin anhydride end-products, crystal structure and solid-state behavior of aspirin anhydride determined

The use of crystallographic data to explain the products of solid-state reactions has provided insight into these reactions and the factors controlling them (1–3). However, few studies have been conducted to determine the relationship between the crystal structure and the degradation products of pharmaceuticals. The aim of this study was to investigate the products of the true solid-state reaction of aspirin anhydride in terms of the crystal packing. It is hoped that such studies will lay the groundwork for understanding drug degradation in the solid state and how to prevent this degradation.

This investigation involved a microscopic study of the behavior of solid aspirin anhydride during heating and determined the crystal structure of aspirin anhydride using single-crystal X-ray techniques. It was found that solid aspirin anhydride liquefies during reaction even at 50°.

EXPERIMENTAL

Preparation of Aspirin Anhydride—Aspirin anhydride was prepared following literature procedures for the reaction of acetylsalicylic acid with ethyl chloroformate (4, 5). When 10 g of acetylsalicylic acid was mixed with 6.2 g of ethyl chloroformate with cooling, ~4 g of crystals formed, mp 79–81°. Subsequent structure determination showed these crystals to be aspirin anhydride.

Crystal Properties of Aspirin Anhydride ($C_{18}H_{14}O_7$, mol. wt. = 342.31)—The crystals were tetragonal with a = 8.457(1), c = 23.166(6) Å, V = 1656.85 Å^3, z = 4, ρ_{calc} = 1.372 g/cm^3, μ (20°, CuKα, λ = 1.5418 Å) = 9.176 cm^{-1}; systematic absences $00l$, $l \neq 4n$; $h00$ $(0k0)$, $h \neq 2n$ $(k \neq 2n)$; space group $P4_12_12$ or $P4_32_12$. The structure was determined assuming space group $P4_12_12$.

Data Collection—A crystal of 0.49 × 0.48 × 0.20 mm^3 was obtained from absolute ethanol by cooling and was aligned with its long axis parallel to the ϕ direction of a Picker four-angle diffractometer. The ϕ direction thus was parallel to the a axis. Intensity data were collected with CuKα radiation and a scintillation counter for the reciprocal octant hkl to a 2θ maximum of 133.85° using a $\theta/2\theta$ scan of 2.4° and a scan speed of 30 sec/degree. Background readings were taken for 10 sec at each end of the scan range. During the data collection (120 hr), the decay of two reference reflections was only 6.7%.

Equivalent reflections in the two semioctants hkl $(h > k)$ and khl $(h < k)$ were averaged using a discrepancy factor R of 0.067. If:

$$\frac{|I(hkl) - I(khl)|}{[I(hkl)I(khl)]^{1/2}} \leq R$$

the average was taken; otherwise, the reflection with the larger net count was retained. The total number of independent reflections was 946, 645 of which satisfied the criterion $F > 3\sigma(F)$ and were considered to be observed. Lorentz polarization corrections were applied to all data. No absorption correction was made.

Structure Determination and Refinement—The 100 reflections of the largest E-forming 843 Σ_2 relationships were sorted with respect to the strength of the phase reliability given by the equation $\sigma_h = 2\sigma_3\sigma_2^{-3/2}|E_h|(s_k^2 + c_k^2)^{1/2}$, where the symbols are defined by Karle and Karle (6). The convergence method was applied to generate 64 starting sets which, upon multiple-tangent refinement, yielded sets of phases with various figures of merit (7). An E-map calculated from the set of phases with the highest figure of merit revealed the positions of 12 of the 13 nonhydrogen atoms. The remaining atom was the oxygen atom.

Full matrix least-squares techniques were used to refine the structure. A weighting scheme ($w^{-1} = (|F_{obs} - B|/A)^2 + 1$, where A = 2.5 and B = 11.3) was also used. Refinement with nonhydrogen atoms proceeded smoothly to an R of 0.105 and a wR of 0.080. A difference Fourier map then revealed the positions of five of the seven hydrogen atoms, and the other two methyl hydrogen atom positions were calculated. The refinement holding the hydrogen atoms invariant with a temperature factor of $B_{overall}$ + 1 then proceeded smoothly to an R of 0.085 and a wR of 0.074. Release of the constraints on the hydrogen atoms resulted in improvement of the R factor to 0.082 and chemically reasonable positions for the aromatic hydrogen atoms but unreasonable positions for the methyl hydrogen atoms; thus, these positions were recalculated, and the final

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