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TECHNICAL MANUSCRIPT 552

SYNTHESIS AND CHARACTERIZATION OF 2-ETHYL-3-HEXANOL p-HYDROXYBENZOATE

John W. E. Brown

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DEPARTMENT OF THE ARMY
Fort Detrick
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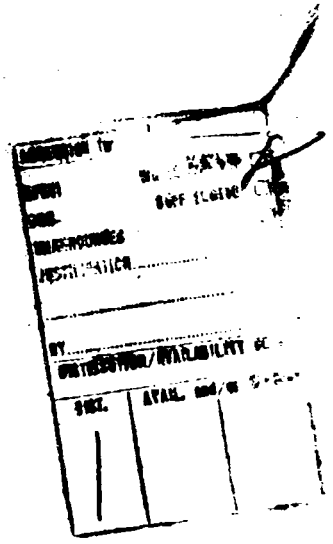

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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ABSTRACT

2-Ethyl-3-hexanol p-hydroxybenzoate was synthesized and characterized by infrared and ultraviolet spectroscopy and thin-layer (TLC) and gas-liquid chromatography (GLC). These methods proved the existence of stereoisomers and allowed the prediction of preferred conformations. The RR and SS enantiomers had a higher boiling point, a lower hR_f value on TLC, and were eluted faster on GLC than the RS and SR diastereoisomers. The ester exhibited antimicrobial activity against gram-positive bacteria and yeast, but not against gram-negative bacteria. The ester also exhibited sunscreen properties and was not toxic to animals.

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I. INTRODUCTION

Methyl, ethyl, propyl, and butyl esters of para-hydroxybenzoic acid are extensively used in the pharmaceutical and food industries as preservatives. These esters, commonly sold under the trade names Parabens* or Parasepts,** have been described as the nearest approach to the ideal pharmaceutical.[1] An increased bacteriostatic and fungistatic activity of these esters has been correlated to an increased chain length of the alcohol substituent.[2-7]

2-Ethyl-1,3-hexanediol contains a primary hydroxyl group that should esterify p-hydroxybenzoic acid more rapidly than the secondary hydroxyl group. The ester thus produced should contain a secondary alcohol group and a relatively long, branched carbon skeleton. Because the diol contains two asymmetric carbon atoms at the 2 and 3 positions, and because no bonds to these atoms would be broken in esterification, four stereoisomers are possible in the diol and ester.

The present study was initiated to (i) synthesize the novel compound 2-ethyl-3-hexanol p-hydroxybenzoate, (ii) determine its properties and action, and (iii) study its stereoisomers.

II. SYNTHESIS OF 2-ETHYL-3-HEXANOL para-HYDROXYBENZOATE

2-Ethyl-1,3-hexanediol was distilled at atmospheric pressure through a 10-inch glass-packed column into low- (up to 242 C) and high-boiling (244 C) components. Unless otherwise stated, this study refers to the synthesis product of the high-boiling component.

0.3 M p-Hydroxybenzoic acid (0.3 M), 2-ethyl-1,3-hexanediol (0.3 M), 200 ml xylene, and 2 ml of phosphoric acid (85%) were refluxed in a Dean-Stark azeotropic distillation apparatus. The reaction mixture was heated to 85±10 C for the first 10 hours and then refluxed at 143 C for 46.75 hours; 0.3 ml and 0.2 ml of additional phosphoric acid were added after 38.75 and 41.75 hours, respectively. The reaction mixture was extracted with 10% $NaHCO_3$ and washed with H_2O; separation was difficult. Xylene was removed by vacuum distillation, and the ester was dissolved in diethyl

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* Trademark for the butyl, ethyl, methyl, and propyl esters of p-hydroxybenzoic acid, Washine Chemical Corporation, 165 Main Street, Lodi, New Jersey, 07644.

** Trademark for group of neutral esters of p-hydroxybenzoic acid; includes the benzyl, butyl, ethyl, methyl, and propyl esters; white powder. Heyden Division, Tenneco Chemicals, Inc., 300 East 42nd Street, New York, New York, 10017.

ether to afford better separation and extracted with 10% NaOH. The extract was acidified with 10% H_2SO_4 and the viscous ester extracted with ether. The ether was evaporated and the ester dissolved in ethanol; carbon was added and the solution warmed to 75 C and filtered. Water was added to incipient cloudiness and the ester extracted with benzene. The benzene was vacuum-distilled and the ester dried 20 hours under vacuum. Recovery equaled 56.95% of the theoretical yield. The viscous, light-brown ester had a boiling point of 243.5 to 244.5 C (Siwoloboff's method), a density of 1.017, and a penetrating odor. The ester was soluble in ether, carbon tetrachloride, ethanol, xylene, and benzene, but insoluble in hexane and water.

III. INFRARED SPECTROSCOPY

Infrared spectra of concentrated and dilute 2-ethyl-3-hexanol p-hydroxybenzoate (Fig. 1) were obtained using a Beckman IR 8 spectrophotometer. A thin smear of the concentrated ester was placed on a salt crystal and the dilute ester in CCl_4 was placed in 1-mm salt cell, and each was analyzed. The results summarized in Table 1 showed weak absorption of the dilute ester at 2.78 μ, indicating a non-bonded -OH group of a phenol. The strong absorption at 2.98 μ corresponded to a hydrogen-bonded -OH group. The strong absorption at 6.23, 6.29, 6.61, and 6.88 μ verified aromatic character, but the absence of any absorption from 3.25 to 3.33 μ and the strong absorption at 3.38, 3.41, and 3.48 μ suggested that the molecule had a predominantly alkane character. These observations were supported by the absorption at 6.94, 7.26, 10.36, 13.05, and 14.41 μ. A para-substituted benzene ring was verified by absorption at 6.47 and 11.81 μ. Absorption at 7.64, 9.00, and 9.13 μ corresponded to C-O stretching of a secondary alcohol; that at 9.00 μ indicated hydrogen bonding, that at 9.13 μ indicated non-bonding. The absorption at 7.89, 8.16, and 8.61 μ supported the C-O stretching of an ester; that at 7.89 and 8.61 μ indicated non-bonding, that at 8.16 μ indicated either hydrogen bonding to the etheral oxygen of the ester or the C-O stretching of a phenol.[8,9] The doublet peak at 5.84 and 5.95 μ indicated a complex interaction of the carbonyl group. The absorption at 5.84 μ corresponded to C=O stretching of an ester hydrogen bonded at the etheral oxygen, while that at 5.95 μ indicated a hydrogen-bonded or conjugated carbonyl system.[8,9]

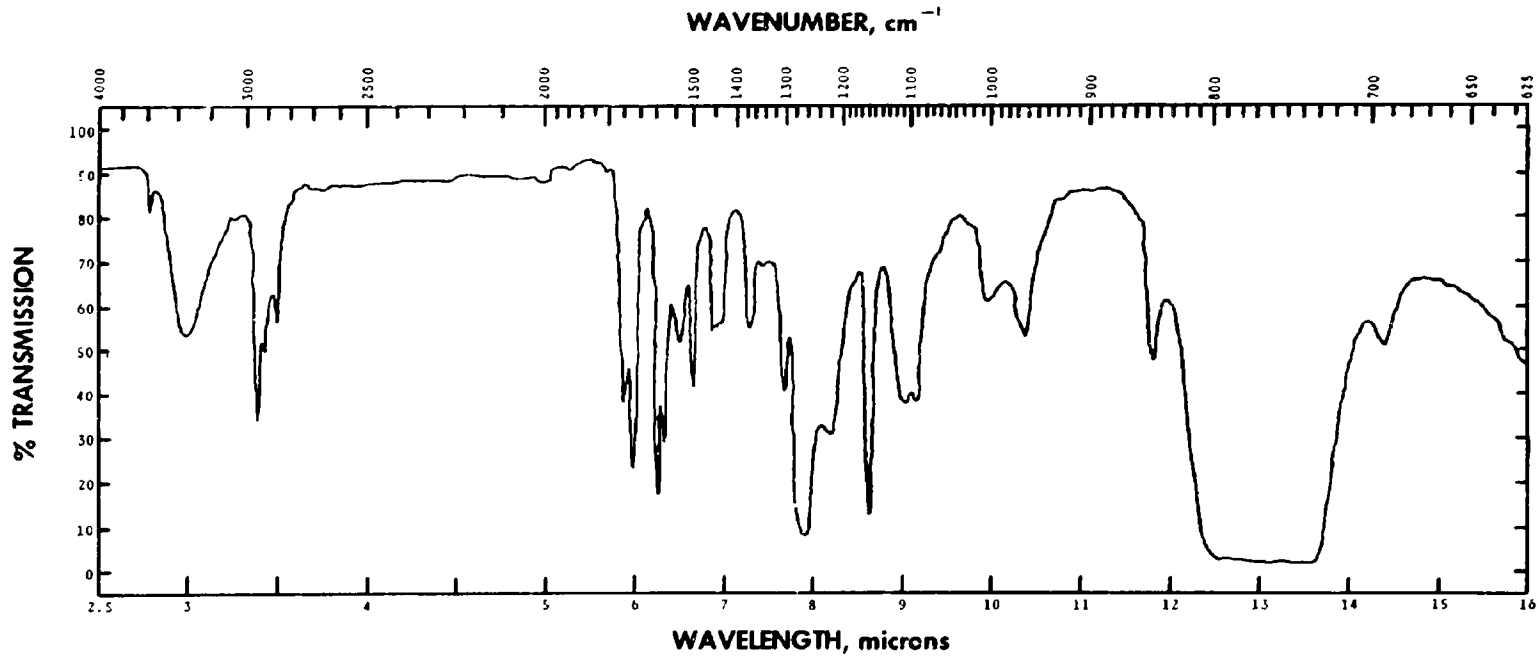

FIGURE 1. Infrared Spectra of 2-Ethyl-3-Hexanol p-Hydroxybenzoate in CCl_4.

TABLE 1. INFRARED ABSORPTION BANDS OF 2-ETHYL-3-HEXANOL p-HYDROXYBENZOATE

Wavelength, μ			
Dilute[a]	Concentrated	Assignment[b]	Reference
2.78	-	O-H (free phenol -OH)	8,9,10
2.98	3.00	O-H (bonded -OH)	8,9,10
3.38	3.39	C-H (alkane $-CH_3$)	8
3.41	3.41	C-H (alkane $-CH_2-$)	8
3.48	3.49	C-H (alkane $-CH_2-$ and $-CH_3$)	8
5.84	(5.86)	C=O (ester H-bonded at C-O)	8,9
5.95	5.97	C=O (H-bonded or conjugated)	8,9,10
6.23	6.23	C=C (phenyl)	8
6.29	6.27	C=C (phenyl, conjugated)	8,10
6.47	-	C=C (phenyl w/para activating and deactivating groups)	10
6.61	6.63	C=C (phenyl)	8
6.88	6.86	C=C (phenyl)	8
6.94	6.95	C-H (alkane $-CH_2-$)	8
7.26	7.28	C-H (alkane $-CH_3$)	8
7.64	7.66	C-O (secondary alcohol)	8
7.89	7.88	C-O (ester not H-bonded)	8,9
8.16	8.16	C-O (ester H-bonded at C-O or C-O of phenol)	8,9
8.61	8.61	C-O (ester)	10
9.00	8.97	C-O (secondary alcohol H-bonded)	8,9
9.13	9.13	C-O (secondary alcohol no H-bond)	8,9
-	9.73	C-C-C (phenyl, uncertain)	10
9.97	9.92	C-O (primary alcohol, impurity)	10
10.36	10.39	C-C (alkane)	10
11.81	11.83	para-substituted benzene	8,9,10
(CCl_4)	13.05	C-H (phenyl)	10
14.41	14.41	C=C-C (phenyl)	10

a. 0.1 M/liter in CCl_4.
b. ( ) indicates shoulder.

The phenomenon of a doublet peak in the infrared carbonyl stretching region has been attributed to the occurrence of (i) an equilibrium between associated and non-associated forms, (ii) an equilibrium of conformers, (iii) Fermi resonance, and (iv) mechanical coupling.[8] Although the observed doublet could be attributed to any or all of the factors cited above, the postulation of equilibrium of conformers appeared to be one of the more probable factors because of stereoisomerism. The stereoisomers of 2-ethyl-1,3-hexanediol are shown in Figure 2. These same conformational isomers are possible for 2-ethyl-3-hexanol p-hydroxybenzoate; structures I and II are enantiomers, and III and IV are enantiomers but diastereoisomers of I and II.

Henbest and Lovell[9] studied the role of hydrogen bonding in four isomers of the cyclic 1,3-diols of 3-acetoxy-5-alcohols of the cholestane and coprostane series. The C=O stretching frequency was raised and the C-O frequency lowered in diaxial compounds conforming to hydrogen bonding. Hydrogen bonding to both C=O and C-O bonds of the esters was observed; bonding to the alcoholic oxygen in the esters was postulated to be due to the formation of a quasi six-membered ring in preference to a quasi eight-membered ring. This same hydrogen bonding situation can exist in the more flexible acyclic isomers of 2-ethyl-3-hexanol p-hydroxybenzoate. The infrared spectrum of the prepared ester was compared with that obtained by Henbest and Lovell.[9] Absorption at 2.78, 2.98, 5.95, 7.89, and 9.13 μ indicated no hydrogen bonding of the ester group, while that at 2.98, 5.84, 8.16, and 9.00 μ corresponded to hydrogen bonding at the C-O group of the ester. Supportive studies were conducted by Kuhn et al.[11,12] and Schleyer.[13] Thus, the infrared spectrum of 2-ethyl-3-hexanol p-hydroxybenzoate supports the postulation of equilibrium of conformers.

2-Ethyl-1,3-hexanediol contains two asymmetric carbon atoms of position C-2 and C-3 and, therefore, can exist as two pair of enantiomers. In Figure 2, structures I and II are a pair of enantiomers that are also diastereoisomers of structures III and IV. These diastereoisomers have different physical properties and, in principle, can be separated. An analogous situation exists for the p-hydroxybenzoic acid ester, because no bond to the asymmetric carbon atoms of the diol would be expected to be broken in forming the ester.

As previously stated, the diol was fractionated by distillation and the high-boiling fraction used to prepare the ester. Intermolecularly hydrogen-bonded molecules have higher boiling points than intramolecular hydrogen-bonded molecules. The structures in Figure 2 were inspected for the most probable conformations allowing hydrogen bonding and were checked with CPK Atomic Models built on a scale of 1.25 cm/Angstrom. Structures I,c, and II,c, provide the least steric repulsion and conform to intermolecular hydrogen bonding. Structures III,a and b, and IV,a and b, also provide the least steric repulsion, but conform to intramolecular hydrogen bonding. In the gas phase, structures I,c, and II,c, would be more stable because of dipole-dipole repulsion. In structures III,c, and IV,c, the dipole-dipole repulsion is sterically hindered, thus lowering the stability of the molecular configuration.

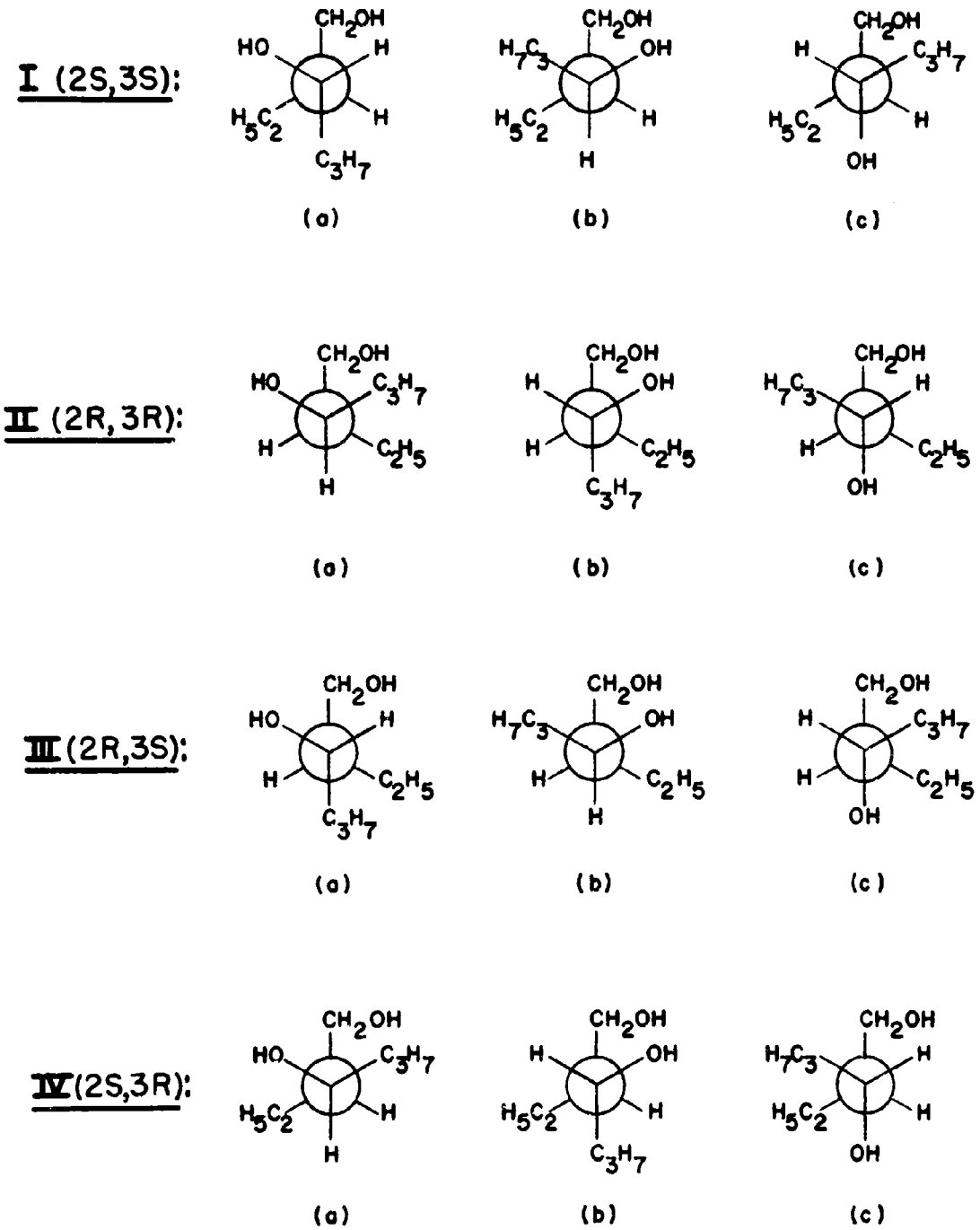

FIGURE 2. Conformations of 2-Ethyl-1,3-Hexanediol.

IV. THIN-LAYER CHROMATOGRAPHY

2-Ethyl-3-hexanol p-hydroxybenzoate was subjected to thin-layer chromatography (TLC) to determine purity and possible separation of isomers. The ester, 2-ethyl-1,3-hexanediol, and p-hydroxybenzoic acid were spotted on TLC silica gel G, plastic plates* activated 4 hours at 105 C. The covered chamber (8.5 x 4 x 10 inches) was lined on all sides with filter paper to provide a highly saturated chamber. The chromatogram was developed 40 min in hexane, diethyl ether, and acetic acid (70:30:2) and produced a 13-cm run. The spots were visualized with I_2 vapor.[14]

Four spots were produced by the ester at hR_f values of 17, 23, 33, and 50. p-Hydroxybenzoic acid and the high-boiling diol each produced a single spot at hR_f 25 and 33, respectively. These corresponded to the hR_f values of 23 and 33 obtained for the ester. The spot obtained from the ester at hR_f 17 was not identified. A chromatogram of the low-boiling diol was obtained concurrently and gave a single spot at hR_f 36. The greater hR_f value of the low-boiling diol was attributed to the fewer number of bonding sites in the molecule because of intramolecular hydrogen bonding. Similar results have been shown for the cyclic 1,3-diols; the cisoid forms have higher hR_f values than the transoid forms.[9,15]

To obtain a large sample for subsequent identification, the ester was chromatographed on 20 x 20 cm glass plates using silica gel G (500 μ) with 10% $CaSO_4$ added as a binder. The TLC plate was activated at 150 C and stored 3 days before use. About 100 mg of the ester in chloroform was streaked on the plate and developed as previously described. The total run was 12 cm in 21 min. The streak was visualized by spraying a 0.5-cm side strip of the TLC with a 5% solution of I_2 in chloroform. Three streaks at hR_f values of 15, 25, and 45 were obtained. The sprayed area was discarded, and the areas containing the separated ester were scraped from the plate and extracted with hot ether, filtered and evaporated. The extracts were identified by gas-liquid chromatography and infrared spectroscopy. The compounds at hR_f 15 and 45 were identified as 2-ethyl-1,3-hexanediol and 2-ethyl-3-hexanol p-hydroxybenzoate, respectively. The compound at hR_f 25 contained an unidentified impurity and 2-ethyl-3-hexanol p-hydroxybenzoate.

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* Eastman Chromatogram Sheet, Type K 301 R, 20 x 20 cm, 250 μ; Eastman Kodak Co., Rochester, N.Y.

V. GAS-LIQUID CHROMATOGRAPHY

Gas-liquid chromatography of 2-ethyl-1,3-hexanediol and 2-ethyl-3-hexanol p-hydroxybenzoate was performed using Programmed Temperature Gas Chromatograph.* The instrument was programmed from 40 to 225 C at 10 C per minute, and the samples were chromatographed on a 6 ft by ¼-inch-O.D. column of OV-17 (3% phenylmethylsilicone on 80- to 100-mesh Chromasorb W-HP).

Eight microliters of a 5% CCl_4 solution of the ester were chromatographed A small peak at 182 C and two large peaks at 140 and 225 C were obtained. The same quantity of the diol and p-hydroxybenzoic acid were chromatographed separately. A small peak at 178 C and a large peak at 140 C were produced by the diol; the acid produced a characteristic hump above 225 C. The peak produced by the ester at 225 C was characterized by comparing with a chromatogram obtained from methyl p-hydroxybenzoate.

As previously reported, a TLC of the ester produced three distinct spots that were analyzed by GLC. The spot at hR_f 15 produced a single peak at 143 C and the spot at hR_f 25 gave a peak at 180 C and a small peak at 225 C. The spot at hR_f 45 produced a single peak at 225 C corresponding to the pure ester.

The trimethylsilyl ether derivatives of compounds containing -OH groups have been extensively studied using GLC.[18-20] The added volatility of silyl ethers enhances the investigation of racemic mixtures. Trimethylchlorosilane (0.1 ml) and hexamethyldisilazane (1.0 ml) were reacted with a 5% solution of the diol in pyridine at 40 C for 4 hr. The reaction mixture was injected into the chromatograph. Trimethylsilyl ether derivatives were prepared for the undistilled diol, the low-boiling diol, and the high-boiling diol. The undistilled diol produced three separate peaks at 140, 142, and 144 C (Fig. 3,A). The low-boiling diol produced a single peak at 144 C and minor inflections at 140 and 142 C (Fig. 3,B). The high-boiling diol produced two peaks of unequal intensity at 140 and 142 C and a minor peak at 144 C (Fig. 3,C). These results and a comparison of the infrared spectra of the three separate fractions confirmed that the isomers of 2-ethyl-1,3-hexanediol were partially resolved.

A quantitative analysis of the fractionated diol was performed by measuring the peak areas obtained from GLC (Table 2). The high-boiling diol contained 9.07% of the low-boiling diol as an impurity, and the low-boiling diol contained 9.50% of the high-boiling diol as an impurity. The average per cent of each peak obtained for the high- and low-boiling diols checked within 3% of the undistilled diol. This difference was insignificant.

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* Model 810, F & M Scientific Corporation, Avondale, Pa.

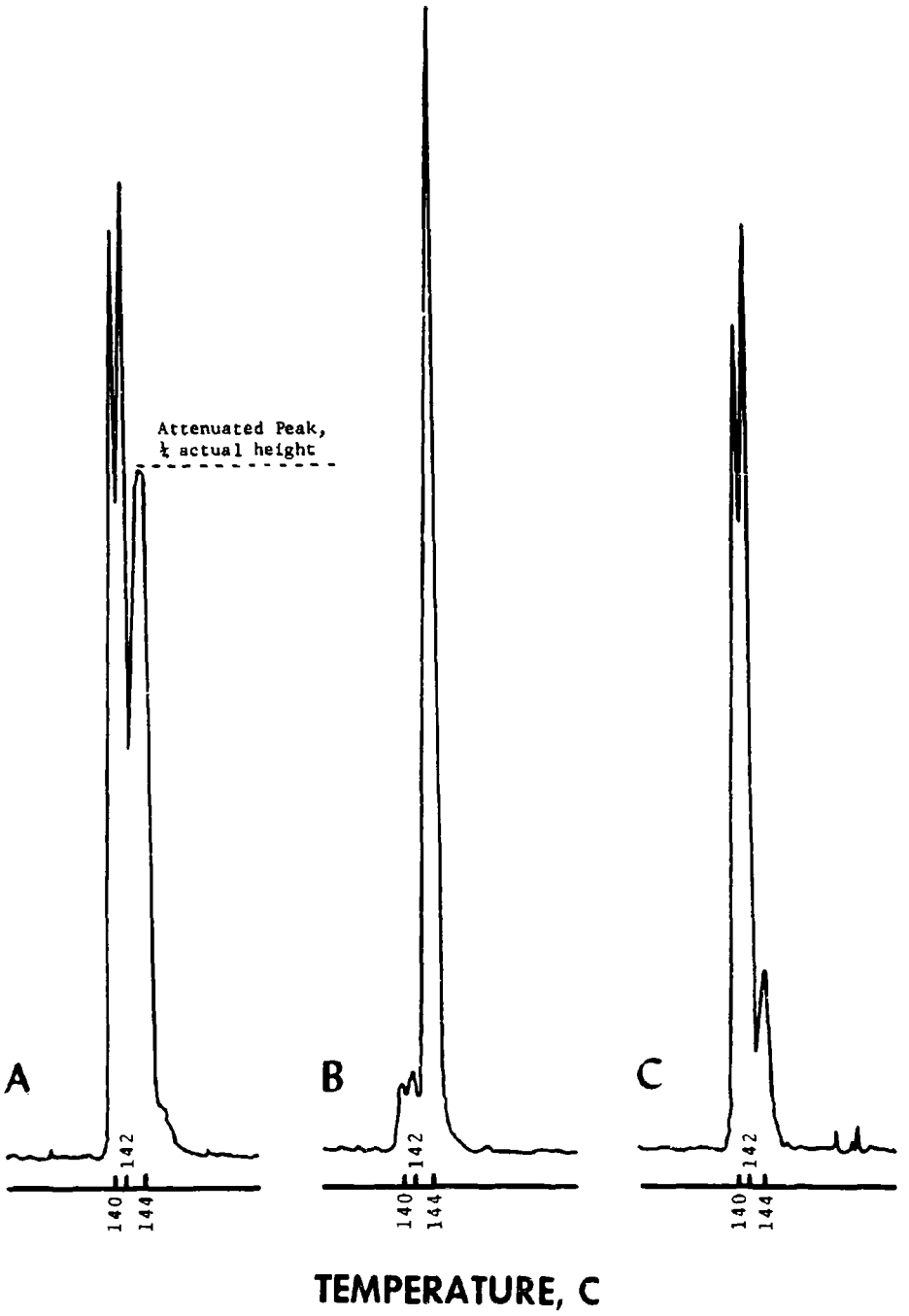

FIGURE 3. Gas-Liquid Chromatograms of Silyl Ether Derivatives of (A) Undistilled, (B) Low-Boiling, and (C) High-Boiling 2-Ethyl-1,3-Hexanediol.

TABLE 2. QUANTITATIVE ANALYSIS OF HIGH- AND LOW-BOILING FRACTIONS AND UNDISTILLED 2-ETHYL-1,3-HEXANEDIOL BY GAS-LIQUID CHROMATOGRAPHY[a]

Fraction	Per Cent of Peak Area/Elution Temperature		
	140 C	142 C	144 C
High-boiling	40.63	50.30	9.07
Low-boiling	4.40	5.10	90.50
Mean ($\bar{X}$)	22.52	27.70	49.78
Undistilled	21.68	25.71	52.61

a. Trimethylsilyl ether derivatives of diol.

These results further clarified the conformation analysis presented in the infrared analysis section. Structures I,c, and II,c, (Fig. 2) were assigned to the high-boiling diol because of intermolecular hydrogen bonding and dipole-dipole repulsion. This same dipole-dipole repulsion in I,c, and II,c, causes a decreased polarity of the entire molecule in comparison with structures III,a and b, and IV,a and b (Fig. 2). It is therefore reasonable to assume that, on the polar OV-17 column, the molecule of lowest polarity will be eluted first. Although the peaks were described as being eluted at different temperatures, this also means that the retention times were different; i.e., the interaction in the gas and liquid phases differed. These results substantiate the work of Gil-Av et al.,[21] who found the RS or SR diastereoisomers of various alcohols to have the higher retention time. However, they could not attribute all similar cases to molecular configuration. Similar results have been obtained with racemic carbohydrates and proteins.[22,23]

The trimethylsilyl ether derivative of the high-boiling diol produced a double peak at 140 and 142 C (Fig. 3,C) with a 9.67% difference in peak area. This difference in peak area indicated that, if the peaks were caused by the optical isomers, the optical activity could possibly be measured. However, polarographic measurements were negative. Based on the assumption presented in the preceding paragraph, the observed doublet may be caused by rotational isomers as I,a and b, and II,a and b (Fig. 2), which would have a polarity different from configurations I,c, and II,c (Fig. 2). The single peak observed for the trimethylsilyl ether derivative of the low-boiling diol would, therefore, indicate a more stable conformation in all type III and IV structures (Fig. 2).

VI. ULTRAVIOLET SPECTROSCOPY

The main ultraviolet absorption band (B-band) of 2-ethyl-3-hexanol p-hydroxybenzoate was investigated. Both hydrogen bonding and steric effects are revealed in the B-band of para-substituted benzoic acid esters; intermolecular hydrogen bonding increases the absorption intensity but not the maximal wavelength. Intramolecular hydrogen bonding has a small effect on the B-band by increasing the wavelength and lowering the absorption, and small steric effects frequently cause only a change in absorption intensity.[24-38]

Model compounds were obtained from the literature to predict the absorption intensity and B-band position. Table 3 shows various benzoic acids and esters containing a para-substituent with a negative inductive effect (i.e., electron attracting) used as models. The -OH group has a stronger negative inductive effect than $-OCH_3$ or -I groups and thus lowers the absorption intensity but has little effect on the wavelength. The phenyl esters of p-methoxy and p-iodobenzoic acids show little difference in wavelength or absorption intensity, and the same would be expected of the p-hydroxy ester. For each micron of wavelength increase, both the phenyl p-methoxy and p-iodobenzoate had an average absorption intensity increase of 577. Therefore, a p-hydroxybenzoic acid ester should have an ultraviolet spectrum at 259 to 261 μ and an absorption intensity of 17,116 to 18,277 in the B-band region in ethanol. The ultraviolet spectra of 2-ethyl-3-hexanol p-hydroxybenzoate and butyl p-hydroxybenzoate were determined in ethanol and CCl_4 (Table 4), using a UV spectrophotometer.* The observed and predicted values for p-hydroxybenzoic acid ester are in agreement.

TABLE 3. ULTRAVIOLET SPECTRA OF para-SUBSTITUTED BENZOIC ACIDS AND ESTERS IN 95% ETHANOL (B-BAND)[24]

	Acids		Phenyl Esters	
para-Substituent	λ max	ε max	λ max	ε max
-OH	251	12,500	-	-
$-OCH_3$	249	14,000	261	22,000
-I	252	17,000	259	21,000

* Spectronic 505, Analytical Systems Division, Bausch & Lomb, Inc., 22968 Bausch Street, Rochester, New York, 14602.

TABLE 4. ULTRAVIOLET SPECTRA OF 2-ETHYL-3-HEXANOL p-HYDROXYBENZOATE AND BUTYL p-HYDROXYBENZOATE IN ETHANOL AND CCl_4

Ester	Band	95% Ethanol		CCl_4	
		λ max	ε max	λ max	ε max
2-Ethyl-3-hexanol	A	207.0	15,460	-	-
	B	259.5	17,420	260.5	6,100
	C	271.7	11,400	271.0	1,700
Butyl	A	209.7	14,140	-	-
	B	258.5	17,560	259.5	5,800
	C	271.7	10,600	271.0	1,200
Predicted	B	259-261	17,116-18,277	-	-

In comparison to the butyl ester, the 2-ethyl-3-hexanol ester was bathochromically shifted one micron in both ethanol and CCl_4 solutions. This shift was attributed to structural changes. A bathochromic shift of one micron was also observed for each ester when the solvent was changed from ethanol to CCl_4. The 2-ethyl-3-hexanol ester displayed a small hypochromic effect in ethanol and a small hyperchromic effect in CCl_4. This hypochromic effect in ethanol indicated a decreased conjugation due to steric interactions of intermolecular hydrogen bonding, and the small hyperchromic effect indicated increased conjugation due to a more stable and planer configuration. C-bands appeared as inflections and A-bands appeared as main absorption peaks in the spectra of both esters. These bands were not analyzed.

The antisunburn or sunscreen activity of the ester was evaluated by ultraviolet spectroscopy. Absorption at 3,080 Angstroms, the peak of the "Sunburn Curve," was determined in 1-cm cells using 1×10^{-2} and 1×10^{-3} M/liter of the ester in ethanol. The results were calculated in terms of a sunscreen index as outlined by Kumler.[30] The sunscreen index of the ester was calculated to be 0.1 to 0.2, indicating that a 40 to 80% solution would effectively shield the skin from rays in the sunburn region (3,080 Angstroms).

VII. ANTIMICROBIAL EVALUATION OF 2-ETHYL-3-HEXANOL p-HYDROXYBENZOATE

Zone inhibition tests were performed to test the antimicrobial activity of 2-ethyl-3-hexanol p-hydroxybenzoate.[31,32] The ester was serially diluted in 95% ethanol, and 0.625-cm-diameter sterile filter paper discs were saturated with the solutions and dried; each disc absorbed approximately 3.5×10^{-3} ml of the solutions.

Pure stock cultures of the test organisms were streaked on nutrient agar plates (Sabouraud's agar for the yeast culture) at a concentration of 1×10^{6} to 1×10^{9} organisms per plate. The cultures were incubated at 37 C, with the exception of Serratia marcescens and Saccharomyces sp., which were incubated at 25 C. Gram-negative bacteria (S. marcescens, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae), gram-positive bacteria (Bacillus subtilis var. niger spores, Sarcina lutea, Staphylococcus aureus) and the yeast Saccharomyces sp. were tested (Table 5).

2-Ethyl-3-hexanol p-hydroxybenzoate demonstrated antimicrobial activity against gram-positive bacteria and yeast organisms, but not toward gram-negative bacteria. B. subtilis var. niger, S. lutea, and S. aureus were inhibited at ester concentrations of 1×10^{-5}, 1×10^{-3} and 1×10^{-2} M/liter, respectively. Only the concentrated ester inhibited the yeast organisms.

Tubes containing 9.9 ml of nutrient broth were inoculated with 1×10^{3} E. coli and B. subtilis and 0.1 ml of an ethanol solution of the ester. This serial dilution provided ester concentrations of 1×10^{-4} to 1×10^{-11} M/liter. Higher concentrations precipitated out of solution. The results are presented in Table 6. Growth of the gram-negative bacteria was retarded during the first 24 hours of growth, but after 72 hours no difference was noted. Growth of the gram-positive bacteria B. subtilis was inhibited at an ester concentration of 1×10^{-4} M/liter up to 72 hours, but inhibited only 24 hours at 1×10^{-5} M/liter.

TABLE 5. INHIBITION OF GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA AND YEAST BY VARIOUS CONCENTRATIONS OF 2-ETHYL-3-HEXANOL *para*-HYDROXYBENZOATE

Concentration		Width of Inhibition Zone Around Disc, mm									
		Gram-Negative[a]		Gram-Positive						Yeast	
	mg/Disc,	S. marcescens		B. subtilis		S. lutea		S. aureus		Saccharomyces sp.	
M/liter	x 9.3[b]	24 hr	72 hr	24 hr	72 hr	24 hr	72 hr	24 hr	72 hr	24 hr	72 hr
Undilute	3.56	8[c]	8[c]	3.0	3.0	4.0	4.5	3.0	3.0	0	0.4
10^{-1}	10^{-1}	3[c]	3[c]	2.5	2.5	3.0	4.0	2.0	2.0	0	0.1
10^{-2}	10^{-2}	2[c]	2[c]	2.0	2.0	2.5	2.5	1.5	1.5	0	0
10^{-3}	10^{-3}	1[c]	1[c]	1.2	1.2	2.0	0.5	1.0	1.0	0	0
10^{-4}	10^{-4}	0.5[c]	0	0.5	0.5	0.7	0.1	0	0.4	0	0
10^{-5}	10^{-5}	0	0	0.5	0.5	0	0	0	0	0	0
10^{-6}	10^{-6}	0	0	1.0	1.0	0	0	0	0	0	0
10^{-7}	10^{-7}	0	0	0	0	0	0	0	0	0	0
10^{-8}	10^{-8}	0	0	1.0	1.0	0	0	0	0	0	0
10^{-9}	10^{-9}	0	0	0	0	0	0	0	0	0	0
10^{-9}	10^{-10}	0	0	0	0	0	0	0	0	0	0
None (control)		0	0	0	0	0	0	0	0	0	0

a. Three other gram-negative bacteria (*E. coli*, *P. vulgaris*, *K. pneumoniae*) were tested, but no inhibition was observed at either 24 or 72 hours at any concentration tested.

b. Except the undilute compound.

c. Growth was not inhibited; rather, the pigment of *S. marcescens* became intensely red around the discs in zones of the extent indicated.

TABLE 6. ANTIMICROBIAL ACTION OF 2-ETHYL-3-HEXANOL p-HYDROXYBENZOATE IN NUTRIENT BROTH CULTURES[a]

Concentration, M/liter	E. coli		B. subtilis	
	24 hr	72 hr	24 hr	72 hr
10^{-4}	±	+	-	-
10^{-5}	±	+	-	+
10^{-6}	±	+	+	+
10^{-7}	±	+	+	+
10^{-8}	±	+	+	+
10^{-9}	±	+	+	+
10^{-10}	±	+	+	±
10^{-11}	±	+	+	+
Control	+	+	+	+

a. + = growth; ± = retarded growth; - = no growth.

VIII. ANIMAL SENSITIVITY STUDY

Swiss-Webster strain mice (18 to 22 g) were injected subcutaneously with 2-ethyl-3-hexanol p-hydroxybenzoate using a 1-ml, 28-gauge syringe. Dimethyl sulfoxide (DMSO), an aprotic solvent, was used as the diluent because it was an excellent solvent and produced no outward manifestations; however, it has produced slight renal damage in experimental animals and man.[33-35] Three mice were injected per treatment, and six mice were used as a control group (Tables 7 and 8). To determine if a sensitivity to the ester had developed, mice in Treatments A, B, C, D, and G were reinjected subcutaneously with 0.05 ml of the 1 M/liter concentration on the 5th day after the initial injection. No gross abnormalities were observed with any of the concentrations tested.

Three human volunteers topically applied the ester to sensitive areas under the arms and on the legs. No reddening of the skin or other outward manifestation was observed. One of the volunteers was very sensitive to similar compounds found in sunscreen preparations.[30,36]

TABLE 7. DOSAGE OF 2-ETHYL-3-HEXANOL p-HYDROXYBENZOATE ADMINISTERED SUBCUTANEOUSLY IN MICE

Treatment	Concentration, M/liter	DMSO, %	Milliliters Injected	Ester dose/mouse	
				mg/20 g	mg/kg
A	Undiluted	-	0.05	50.85	2,542.5
B	1	74	0.10	26.60	1,331.6
C	0.5	95	0.10	13.30	665.8
D	10^{-1}	90	0.10	2.70	133.2
E	0.5×10^{-1}	95	0.10	1.30	66.6
F	10^{-2}	90	0.10	0.30	13.3
G	10^{-3}	90	0.10	0.03	1.3
H	Control	100	0.10	-	-

TABLE 8. ABNORMALITIES IN MICE INJECTED WITH 2-ETHYL-3-HEXANOL p-HYDROXYBENZOATE IN DMSO[a]

Treatment	Reaction at Indicated Time After Injection		
	1 to 3 Days	4 to 6 Days	7 to 9 Days
A	Large node (3/3)	Small abscess (1/3) Small node (2/3)	Small node (1/3) "Normal" (2/3)
B	Large node (3/3)	Infected eye and node (1/3), node and abscess (2/3)	"Normal" (3/3)
C	Large node (3/3)	Abscess (3/3)	"Normal" (3/3)
D	Large node (3/3)	Abscess (3/3)	Small node (1/3) "Normal" (2/3)
E	Large node (3/3)	Abscess (3/3)	"Normal" (3/3)
F	Large node (3/3)	Abscess (3/3)	"Normal" (3/3)
G	Large node (3/3)	Abscess (3/3)	"Normal" (3/3)
H	Large node (3/6)	One died Abscess (5/5)	"Normal" (5/5)

a. ( ) = mice responding/total tested.

IX. MOSQUITO REPELLENCY STUDY

2-Ethyl-1,3-hexanediol has been used for many years as a mosquito repellent sold under the trade names "6-12," Rutgers 612, or Ent-375. Because 2-ethyl-3-hexanol p-hydroxybenzoate was synthesized from this diol, the repellency of the ester was tested against the mosquito Aedes aegypti. The test methods followed those reported by Bar-Zeev and Ben-Tamar.[57] Cloth squares, each with a 5-mm hole in the center, were wetted with one ml of a 10% ethanol solution of either the high-boiling, low-boiling, or undistilled 2-ethyl-1,3-hexanediol or 2-ethyl-3-hexanol p-hydroxybenzoate. A control was treated with one ml of ethanol. Each cloth was fastened on top of 1-pint ice-cream carton lined with paper toweling and filled three-fourths full of water. Thus, the female mosquitoes were forced to enter the oviposition sites through the hole in the treated cloth. Two days after the mosquitoes were offered a blood meal (guinea pig), the oviposition sites were placed in the cage and left there 3 days. Then, another blood meal was offered to the approximately 2,000 female mosquitoes, and new oviposition sites were placed in the cage for another 3 days.

No differences were noted in the number of eggs oviposited in the sites treated with the three 2-ethyl-1,3-hexanediol fractions or 2-ethyl-3-hexanol p-hydroxybenzoate. In each of the two tests, the control site contained the greatest number of eggs.

X. CONCLUSIONS

2-Ethyl-3-hexanol p-hydroxybenzoate was synthesized by simple acid-catalyzed esterification methods, using p-hydroxybenzoic acid and 2-ethyl-1,3-hexanediol. This method produced a 56.95% recovery of the light-brown, viscous ester. The ester had a boiling point of 243.5 to 244.5 C, a density of 1.017, and was soluble in ether, ethanol, CCl_4, xylene, and benzene, but insoluble in hexane and water.

Infrared spectroscopy of the ester provided proof of the molecular structure. A detailed analysis of the O-H, C=O and C-O infrared stretching frequencies proved intramolecular and intermolecular hydrogen bonding interactions. This analysis supported the postulation of an equilibrium of conformational isomers.

Stereoisomers of 2-ethyl-3-hexanol p-hydroxybenzoate were studied using 2-ethyl-1,3-hexanediol as a model. The diol was distilled into high- (244 C) and low-boiling (242 C) fractions and identified by infrared spectroscopy as 2-ethyl-1,3-hexanediol. The high-boiling fraction was assigned configurations I,c, and II,c, (Fig. 2) because these structures allowed intermolecular hydrogen bonding, provided the least steric repulsion, and allowed greater molecular stability because of the dipole-dipole repulsion in the gas phase. The low-boiling diol fraction was assigned structures III,a and b, and IV,a and b (Fig. 2), because these configurations provided the least steric repulsion and allowed intramolecular hydrogen bonding. However, once in the gas phase, these molecular configurations become unstable because of dipole-dipole repulsion.

The ester was purified by thin layer chromatography, and spots were identified by gas-liquid chromatography and infrared spectroscopy. The diol produced different hR_f values on TLC as a result of conformational configurations. The low-boiling diol allowed intramolecular hydrogen bonding and had the highest hR_f value.

Gas-liquid chromatography of the trimethylsilyl ethers of 2-ethyl-1,3-hexanediol proved the existence of stereoisomers. Quantitative analyses of the high- and low-boiling diols were performed by measuring peak areas. These studies confirmed the previous structural conformation assignments based on polarity differences. However, no definite conclusion could be drawn about the doublet peak observed for the high-boiling diol.

The ultraviolet spectrum for 2-ethyl-3-hexanol p-hydroxybenzoate was accurately predicted by analyzing spectra of similar compounds. The ultraviolet spectrum of a model compound, butyl p-hydroxybenzoate, was compared with that of the ester; differences in absorption intensity and wavelength displacement were attributed to structural changes and differences in conjugation of the two systems.

2-Ethyl-3-hexanol p-hydroxybenzoate exhibited antimicrobial activity against gram-positive bacteria and yeast, but not against gram-negative bacteria. Dilutions of 1×10^{-5} M/liter produced antimicrobial activity.

Mice injected subcutaneously with concentrated and dilute 2-ethyl-3-hexanol p-hydroxybenzoate exhibited no outward deleterious manifestations. The ester dosage ranged from 1.3 to 2,542.5 mg/kg live weight. The ester applied topically to the arms and legs of human volunteers also produced no outward manifestations.

The mosquito repellency properties of the 2-ethyl-3-hexanol ester were as effective as those of the original 2-ethyl-1,3-hexanediol.

The use of 2-ethyl-3-hexanol p-hydroxybenzoate in cosmetics and sunscreen compounds was shown to be feasible.

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13. ABSTRACT

> 2-Ethyl-3-hexanol p-hydroxybenzoate was synthesized and characterized by infrared and ultraviolet spectroscopy and thin-layer (TLC) and gas-liquid chromatography (GLC). These methods proved the existence of stereoisomers and allowed the prediction of preferred conformations. The RR and SS enantiomers had a higher boiling point, a lower hR_f value on TLC, and were eluted faster on GLC than the RS and SR diastereoisomers. The ester exhibited antimicrobial activity against gram-positive bacteria and yeast, but not against gram-negative bacteria. The ester also exhibited sunscreen properties and was not toxic to animals.

14. Key Words

2-ethyl-3-hexanol para-hydroxybenzoate
2-ethyl-1,3-hexanediol
para-hydroxybenzoic acid
esterification
stereoisomers
enantiomers
diastereoisomers
bacteriostasis
fungistasis
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