Concentration, M	H_3	H_5	H_{10}	2-CH_3	4-CH_3	$H_{6,7,8,9}$
3.4×10^{-2}	7.02	8.38	8.53	2.69	d, 2.76, $J = 1.2$ Hz	m, 7.44 and 7.98
2.74×10^{-1}	6.9	8.24	8.48	2.64	2.64	m, 7.42 and 7.98
6.4×10^{-1}	6.77	8.14	8.43	2.57	d, 2.50, $J = 1$ Hz	m, 7.36 and 7.86
Benzene-d_6	5.8	8.20	8.89	2.58	d, 2.27, $J = 0.66$ Hz	m, 7.25 and 7.8

[a] Except as noted, spectra were run in carbon tetrachloride; 1% tetramethylsilane was utilized as the internal standard ($\delta = 0.0$) in all samples.

(2). The chemical shifts for the protons of I at various concentrations are shown in Table I.

Compound I, 150 mg, was refluxed for 15 min in 4 ml of methyl alcohol-d_1, to which had been added 200 mg of metallic sodium. The reaction was then treated with 25 ml of water, the mixture was extracted with ether, and this extract was dried with magnesium sulfate. The residue remaining after evaporation under vacuum was recrystallized from petroleum ether, mp 92–93° [lit. (2) mp 92–93°], and the NMR spectrum was determined in benzene-d_6. The downfield methyl resonance contained 26% less protons than the upfield 4-methyl group.

2-Methyl-4-phenylbenzo[g]quinoline (II) was prepared as described by Huisgen (3). The product melted at 110° [lit. (3) mp 110°]; NMR: δ 8.58 (s, 1H), 8.27 (s, 1H), 8.14 (m, 9H), 7.09 (s, 1H), and 2.76 (s, 3H). No appreciable change in the spectrum was found when the concentration of II was changed.

DISCUSSION

The lack of coupling between proton H_3 and the methyl group of 2-methylquinoline and the fact that the methyl protons of 4-methylquinoline were observed as a doublet ($J = 0.95$ Hz) (4) supported the discussed assignment of the methyl resonances of I. In addition, the methyl resonance of II was a singlet. To show that the doublet of I was the result of coupling to proton H_3 rather than to a long-range interaction, proton H_3 was irradiated to produce a singlet for the 4-methyl resonance of I. The ability of benzene to produce larger upfield chemical shifts for methyl groups on the 4-position of quinoline or pyridine is well documented (1), and the same physical phenomenon should result in an upfield shift for the 4-methyl of I.

When the spectrum of I was run in benzene-d_6, the methyls appeared as expected as a singlet at δ 2.58 and as a doublet at δ 2.27 ($J = 0.66$ Hz). When I was treated with sodium methoxide–methyl alcohol-d_1, the downfield resonance as observed in benzene-d_6 exchanged more rapidly than the upfield resonance, showing that the downfield resonance in benzene-d_6 was due to the more acidic 2-methyl group (5).

The finding of Clar and MacKay (6) that the splitting between methyl-substituted anthracenes and aromatic protons was directly related to the extent of double bond fixation has application to the benzo[g]quinoline system. Since the resonance energy of benzo[g]quinoline is 21.2 kcal/mole less than anthracene, the possibility of even greater bond localization exists with I than with anthracene. The lack of coupling between proton H_3 and the 2-methyls of I and II substantiated the localization of bonds in the hetero ring of benzo[g]quinolines as shown in Structures I and II.

The localization of the bonds in the hetero ring results in less paramagnetic shielding for the 2-methyl group because it is removed from the aromatic naphthalene ring. The 4-methyl group is affected by the paramagnetic shielding of the naphthalene section of the molecule, resulting in its downfield position with respect to the 2-methyl in dilute solutions where solute–solute interactions are minimized. In more concentrated solutions, increased shielding of the 4-methyl group due to solute–solute interactions produces a spectrum that conforms to the classical absorption pattern.

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Quantitative Determination of Phenol by High-Pressure Liquid Chromatography

JOSEPH D. MUSTO, JAYANT N. SANE, and VICTOR D. WARNER [x]

Abstract □ High-pressure liquid chromatography was used with a 5-μm silica gel column to quantitate the phenol in phenolated calamine lotion USP and a commercial antiseptic solution. This method requires less than 10 min/assay, and other compounds present in the products analyzed did not interfere.

Keyphrases □ Phenol—high-pressure liquid chromatographic analysis, pharmaceutical preparations □ High-pressure liquid chromatography—analysis, phenol in pharmaceutical preparations □ Antipruritics—phenol, high-pressure liquid chromatographic analysis in pharmaceutical preparations

Methods for the quantitative analysis of phenol include a bromometric analysis (1), a colorimetric analysis based on the reaction of phenol with copper sulfate (2), and, most recently, a colorimetric analysis based on the reaction of phenol with ferric chloride (3). This report describes a rapid quantitative method for the analysis of the phenol

Table I—Assay Results (Percent Concentration of Phenol) on Phenolated Calamine Lotion and a Commercial Antiseptic Solution

Assay	Phenolated Calamine Lotion		Commercial Antiseptic Solution	
	Assay	USP	Assay	Reported[a]
1	1.01	1.00	1.36	1.4
2	1.02	1.00	1.34	1.4
3	0.96	1.00	1.35	1.4
4	1.02	1.00	1.35	1.4
SD	0.03		0.008	
CV	3.0%		0.59%	
Average deviation	0.02		0.005	

[a] Data from "Handbook of Non-Prescription Drugs," G. B. Griffenhagen and L. L. Hawkins, Eds., American Pharmaceutical Association, Washington, D.C., 1973, p. 130.

present in commercial products using high-pressure liquid chromatography (HPLC).

EXPERIMENTAL

Reagents and Chemicals—All chemicals and reagents were USP, NF, ACS, or chromatographic grade. Phenolated calamine lotion USP (1% phenol) and a commercial antiseptic solution (1.4% phenol) were purchased locally.

Preparation of Solutions—Solutions of 1.00% (w/v) phenol and 1.00% (w/v) hexylresorcinol were prepared in methylene chloride. Five standard solutions were prepared by placing 2 ml of the 1.00% hexylresorcinol in each of five 10-ml volumetric flasks, adding varying amounts of 1.00% phenol (0.5–2.0 ml) to each flask, and diluting to 10 ml with methylene chloride. These dilutions yielded five standard solutions of phenol ranging from 0.050 to 0.200% with the internal standard (0.200% hexylresorcinol). These solutions were used to establish the accuracy and linearity of the chromatographic response. An aqueous standard containing 0.110% (w/v) phenol was used as the primary standard for analysis of the unknown samples.

Establishment of Linearity—The five standard phenol–hexylresorcinol solutions (0.050–0.200%) in methylene chloride served to establish the linearity of response. Ten microliters of each solution was injected into the chromatograph, and the peak height ratio of the phenol to the internal standard (0.200% hexylresorcinol) was calculated and plotted *versus* the phenol concentration. The data indicated that the chromatographic response was linear up to the highest phenol concentration (0.200%) tested.

Assay Procedure—The phenolated calamine lotion was thoroughly mixed. A 10-ml aliquot of the emulsion was diluted to 100 ml with distilled water, and 10 ml of this diluted sample was centrifuged. An aliquot of 5 ml of the supernate was extracted by adding 1.0 ml of 1 N HCl, 4 ml of methylene chloride, and 1 ml of the internal standard (1.00% hexylresorcinol in methylene chloride). The mixture was centrifuged, and the aqueous layer was discarded. Ten microliters of the methylene chloride extracts was injected into the chromatograph.

The commercial antiseptic solution, 10 ml, was diluted to 100 ml with distilled water. A 5-ml aliquot of the diluted solution was extracted by adding 1.0 ml of 1 N HCl, 4 ml of methylene chloride, and 1 ml of the internal standard (1.00% hexylresorcinol in methylene chloride). The mixture was centrifuged, and the aqueous layer was discarded. Ten microliters of the methylene chloride extract was injected.

The aqueous phenol standard containing 0.110% phenol was extracted by adding 1.0 ml of 1 N HCl, 4 ml of methylene chloride, and 1 ml of the internal standard (1.00% hexylresorcinol in methylene chloride). The mixture was centrifuged, and the aqueous layer was discarded. Ten mi-

croliters of the methylene chloride extract was injected. The final concentration of the internal standard in the aqueous phenol standard extract and sample extracts was 0.200%.

The 0.100% phenol standard solution containing 0.200% internal standard (hexylresorcinol in methylene chloride) was always injected during the analysis to monitor instrument response.

The percent phenol in the analyzed sample (% S) was calculated according to:

$$\% S = \frac{R_s}{R_{std}} \% \text{ std } D \qquad (Eq. 1)$$

where % std is the percent phenol in the aqueous phenol standard solution, R_s is the ratio of the peak height of phenol and hexylresorcinol in the extracted sample, R_{std} is the ratio of the peak height of phenol and hexylresorcinol in the extracted aqueous phenol standard solution, and D is the dilution factor (= 10).

Interference from Other Components—In the analysis of phenolated calamine, no interference was observed in the chromatogram. The commercial antiseptic solution contained thymol and menthol in addition to phenol, but neither agent interfered with the assay. Thymol eluted at 1.97 min; menthol, as expected, was not observed by the UV detector.

Instrument Parameters—A high-pressure liquid chromatograph[1] with a UV detector (254 nm) was used with a solvent system containing 63% heptane, 34% chloroform, and 3% methanol. The column[2] was 0.63 cm (1/4 in.) o.d., 15 cm long, and packed with 5-μm silica gel[3]. The temperature was ambient, and the solvent flow was 3 ml/min (at an inlet pressure of 1000 psi). Samples and standards of 10 μl were injected, giving an elution order of phenol (2.75 min) and hexylresorcinol (4.73 min).

DISCUSSION

The results (Table I) show that phenol can be readily assayed in phenolated calamine lotion USP and a commercial antiseptic solution using a simple HPLC method. The extraction and HPLC analyses gave a linear response with phenol concentrations between 0.050 and 0.200%. An aqueous phenol standard containing 0.110% phenol was assayed with each batch of samples, and the concentrations of the samples were calculated based upon this standard. This aqueous phenol standard very closely simulated the unknown samples and was carried through the identical extraction procedure.

A control was also run with each batch to monitor instrument conditions. This procedure minimized assay errors due to technique. Total analysis time per sample was less than 10 min.

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[1] Waters Associates model ALC 202.
[2] Hi-Eff Micropart.
[3] Applied Sciences Laboratories.