Determination of the Chemical Constituents and Spectral Properties of Commercial and NF Reference Standard Potassium Guaiacolsulfonate: Implications of the Findings on Compendial Analytical Methodology

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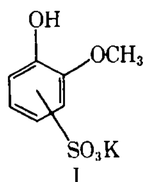
Received June 1, 1983, from the *Department of Medicinal Chemistry/Pharmacognosy, School of Pharmacy, University of Maryland, Baltimore, MD 21201.* Accepted for publication September 26, 1983.

Abstract □ HPLC analysis confirmed a difference in the chemical composition of commercial *versus* NF reference standard potassium guaiacolsulfonate. After separation by fractional crystallization, the two constituents comprising the former sample were identified by ^{1}H-NMR as potassium guaiacol-4- and -5-sulfonate, respectively. The former isomer predominated. The reference standard material was identified as the 4-sulfonate salt by HPLC, NMR, IR, and UV. This difference raises questions concerning the validity of the compendial identification tests and assay procedure for the drug, which are based on methods requiring direct comparison between it and the reference standard. In fact, the IR test has now been shown to be unreliable as a rigorous criterion for identification, in light of significant differences found in the fingerprint regions of the spectra of the two isomers. However, because the two isomers exhibit identical UV absorption characteristics in solutions of pH ≤7 (but not in alkaline solution), the compendial UV identification test and assay procedure appear to be valid under the conditions specified. The pK_a values of the isolated isomers were estimated by a spectrophotometric method to be 8.74 and 9.16 for the phenolic group of potassium guaiacol-4- and -5-sulfonate, respectively.

Keyphrases □ Potassium guaiacolsulfonate—chemical constituents, spectral properties, commercial and NF reference standard □ Isomers—potassium guaiacolsulfonate, determination of chemical constituents and spectral properties, commercial and NF reference standard

Since the first reported synthesis of potassium guaiacolsulfonate *via* sulfonation of guaiacol with concentrated sulfuric acid (1), there has been disagreement over product composition. Some workers have suggested that a single product is formed (2-4); however, others have described isomeric mixtures (1, 5-8). The identification of the mixture as the 1-hydroxy-2-methoxybenzene-4- and -5-sulfonic acid salts by Paul (5) has been accepted (9, 10) and confirmed by others who examined both commercial samples and formulated drug products (11-13).

However, discrepancies in several drug listings (14-16) have persisted. The National Formulary (NF) has failed to be specific about the composition of the officially recognized drug entity (I) with no reference to a mixture of isomers (17). The original analytical methodology introduced in NF XII appears to be based on methodology (18) which (*a*) failed to take into account the fact that the drug may be an isomeric mixture and (*b*) proposed a reference standard prepared by recrystallization of the commercial sample from water or alcohol, which could lead to a product of uncertain composition, since solubility differences between the isomeric components of the mixture of potassium guaiacolsulfonates are known to exist (9, 11).



Although potassium guaiacolsulfonate (I) is no longer included in the official compendium, its commercial use in cough and cold remedies as an expectorant is still widespread. In this study, a commercial sample and an NF reference standard of potassium guaiacolsulfonate are characterized, and the NF XIII methodology (17) is evaluated.

EXPERIMENTAL SECTION

Materials—Commercial[1] and NF reference standard[2] potassium guaiacolsulfonate were used as received. All solvents and standard chemicals employed were reagent grade.

High-Performance Liquid Chromatography—The apparatus consisted of a single-piston reciprocating solvent delivery pump with integral pulse dampener[3]; a valve injector[4] fitted with a 25-μL sample loop; a 30 cm × 4-mm stainless steel column packed with a chemically bonded octadecylsilane reverse-phase packing[5]; a variable-wavelength UV–visible spectrophotometer[6] operated at 278 nm and fitted with an 8-μL, 1-cm path length, quartz flow cell[7] as detector; and a recorder[8].

The mobile phase consisted of 0.005 M tetrabutylammonium hydroxide, adjusted to pH 7.15 with 86% H_3PO_4, and contained 10% (v/v) methanol. It was deaerated by stirring under reduced pressure. The flow rate was maintained at ~1.4 mL/min.

All solutions of potassium guaiacolsulfonate analyzed by HPLC were prepared at a concentration of ~1.3 mg/mL in water. Analysis of the commercial sample of I under the conditions described above resulted in a chromatogram with two baseline-resolved peaks (R_s = 1.7) at retention times (t_R) near 11 and 14 min, respectively, in the area ratio[9] 1.5:1 (Fig. 1). Analysis of the NF reference standard of I under identical conditions yielded a chromatogram containing only a single peak with a retention time of 11 min, which was shown by mixture analysis to be identical with the t_R of the earlier-eluting (major) peak of the commercial sample.

Spectrometric Measurements—^{1}H-NMR spectra were recorded[10] in D_2O[11] containing 1% sodium 3-trimethylsilyl-1-propanesulfonate (DSS)[12]. To generate the phenolate anions of the I isomers, a few drops of NaOD solution[13] were added to the D_2O solutions. Chemical shifts are reported as δ values in parts per million relative to DSS.

IR spectra[14] were run as KBr pellets. UV spectra[15] were recorded at a concentration of 0.02 mg/mL in water, 0.1 M HCl, and pH 7.0 potassium phosphate buffer and at 0.01 mg/mL in 0.1 M NaOH. Molar absorptivities (ε) of the isomers at the wavelengths of maximum absorption in the pH 7.0 phosphate buffer solution were determined from the slopes of Beer's law plots derived from absorbance measurements of seven solutions over the concentration range 8×10^{-5} to 3×10^{-4} M.

[1] ICN Pharmaceuticals, K & K Labs Division, Plainview, N.Y.
[2] USP authentic substance, Lot F; U.S. Pharmacopeial Convention, Inc., Rockville, Md.
[3] Milton Roy Co., Laboratory Data Division, Riviera Beach, Fla.
[4] Valco Instruments Co., Houston, Tex.
[5] μ-Bondapak C_{18}; Waters Associates, Milford, Mass.
[6] Unicam SP1800; Pye-Unicam Ltd., Cambridge, England.
[7] Hellma Cells, Inc., Jamaica, N.Y.
[8] Model EU-20B; Heath Co., Benton Harbor, Mich.
[9] Peak areas were calculated from the product of peak height times the width of the peak at half height.
[10] Model T-60A NMR spectrometer; Varian Instruments.
[11] 99.75 atom% of deuterium; J. T. Baker Chemical Co., Phillipsburg, N.J.
[12] Norell Chemical Co., Landisville, N.J.
[13] 40% in D_2O; Stohler Isotope Chemicals, Rutherford, N.J.
[14] Model IR8 IR spectrophotometer; Beckman Instruments.
[15] Model DB spectrophotometer with laboratory recorder; Beckman Instruments.

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Preparative Isolation of the Constituents of Commercial I by Fractional Crystallization—The commercial sample (90 g) was dissolved in 120 mL of boiling water, and the solution was filtered while hot, cooled to room temperature, and refrigerated overnight. The resulting white crystals were removed by filtration and recrystallized from water to give isomer A (41 g), which has previously been described as the "less-soluble" salt (9).

To the mother liquor (80 mL) from the aforementioned first crystallization was added slowly with stirring 95 mL of absolute ethanol. A white solid precipitated (4 g), which was removed by filtration. This material was purified by suspending in boiling absolute ethanol (125 mL) and then adding sufficient aqueous ethanol (25% ethanol-water), while heating, to completely dissolve the solid. The solution was cooled and allowed to stand overnight. The resulting precipitate was removed by filtration and washed with aqueous ethanol, and then absolute ethanol, to give (after drying) 2 g of isomer B, the "more-soluble" salt.

1-*O*-Acetyl Derivatives of the Isomeric Potassium Guaiacolsulfonates—Each of the aforementioned isomers A and B (200 mg, 0.8 mmol) was refluxed with 10 mL of acetic anhydride and 2 mL of pyridine for 30 min and then cooled. The white crystals were removed by filtration, washed with acetone, and dried, to give 160 mg of potassium 4-(acetyloxy)-3-methoxybenzenesulfonate (acetylated isomer A) [IR(KBr): 1760 cm^{-1} (C=O); ^{1}H-NMR (D_2O): δ 2.36 (s, CH_3CO), 3.92 (s, CH_3O), 7.25 (d, ArH), 7.51 (d of d, ArH), and 7.55 ppm (d, ArH)] and 150 mg of potassium 3-(acetyloxy)-4-methoxybenzenesulfonate (acetylated isomer B) [IR(KBr): 1760 cm^{-1} (C=O); ^{1}H-NMR (D_2O): δ 2.36 (s, CH_3CO), 3.86 (s, CH_3O), 7.24 (d, ArH), 7.60 (d, ArH), and 7.79 ppm (d of d, ArH)].

Anal.[16]—Calc. for $C_9H_9O_6SK$: C, 38.02; H, 3.19; S, 11.28. Found for acetylated isomer A: C, 37.72; H, 3.47; S, 11.21. Found for acetylated isomer B: C, 37.75; H, 3.41; S, 10.99.

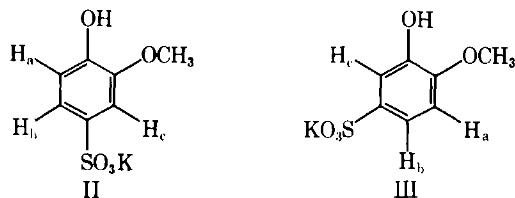
Spectrometric Estimation of the pK_a Values of the Isomers of I—Eleven solutions of each isomer were prepared (0.02 mg/mL), covering the pH range of 5.6–10.0, with 0.1 M potassium phosphate and sodium borate buffer systems. The analytical wavelengths employed were selected by comparison of the spectra of the free phenol and phenolate anion forms of the isomers in pH 5.6 buffer and 0.1 M NaOH solution, respectively, with 257 nm being chosen for isomer A and 296 nm for isomer B. From absorbance measurements of each of the 11 solutions and for the pure anion and free phenol form of each isomer at the selected wavelengths, the ratio $[A^-]/[HA]$ was calculated (19) at each pH[17]. The pK_a of the phenol group was then determined graphically (linear regression analysis) as the *y*-intercept of the linear plot of pH *versus* log $[A^-]/[HA]$ ($r > 0.99$, with unit slope for each determination).

RESULTS AND DISCUSSION

The results of paired-ion HPLC analysis of the commercial and NF reference standard samples of I clearly demonstrate the dissimilarity in their chemical compositions. Furthermore, determination of the retention times of the isolated pure isomers A and B show that the former represents the major constituent in the commercial sample and the sole constituent of the NF reference standard I, whereas isomer B represents the later-eluting peak in the chromatogram of the commercial sample. The structural identities of the two isomers then remain to be established.

Structure Determination of the Isomers of I by ^{1}H-NMR—The aromatic region of the ^{1}H-NMR spectra of isomers A and B both featured an upfield *ortho*-split doublet (H_a, δ 7.00, J = 9 Hz) and a lower-field doublet of doublets (H_b, J_{ortho} = 9 Hz, J_{meta} = 2 Hz) overlapping a *meta*-split doublet (H_c, J = 2 Hz). This pattern of absorption bands establishes that the three substituents on the aromatic ring of both isomers must be oriented asymmetrically [1,2,4 (II) and 1,2,5 (III)] rather than vicinally (1,2,3 or 1,2,6).

In order to assign structures II and III specifically to isomers A and B, respectively, the positions of the three ring protons relative to the ring substituents need to be differentiated for each compound. This was accomplished by determining the effect on the chemical shifts of the ring protons of changing the electronic character of the hydroxyl group of each of the isomers by converting it to its phenoxide ion and acetyl derivative. It is known that the chemical shifts of ring protons *ortho* and *para* to the hydroxyl group are most affected by such changes and *meta* protons are affected less (20). For the derivatives of isomer A, the greatest chemical shift changes were observed for the high-field doublet due to H_a (0.38 ppm upfield in the phenoxide ion, 0.25 ppm downfield in the acetate ester), with the corresponding changes for the absorptions due to H_b and H_c being approximately half as large. For the derivatives of isomer B, the greatest chemical shift changes were observed for the absorptions due to H_b and H_c (0.41 and 0.37 ppm upfield, respectively, in the phenoxide ion; 0.45 and 0.30 ppm downfield, respectively, in the acetate ester), with the corresponding changes in the high-field doublet due to H_a being much smaller (0.09 ppm upfield and 0.22 ppm downfield in the respective derivatives). These results leave no doubt that for isomer A, H_a must be *ortho* to the hydroxy group (*i.e.*, the 6-position) and that for isomer B, H_b and H_c must be *para* and *ortho*, respectively, to the hydroxy group (*i.e.*, the 4- and 6-positions, respectively) (20). Therefore, isomer A, the "less-soluble" salt which represents the earlier-eluting (major) HPLC peak from the commercial sample is potassium guaiacol-4-sulfonate (II) and isomer B is potassium guaiacol-5-sulfonate (III).



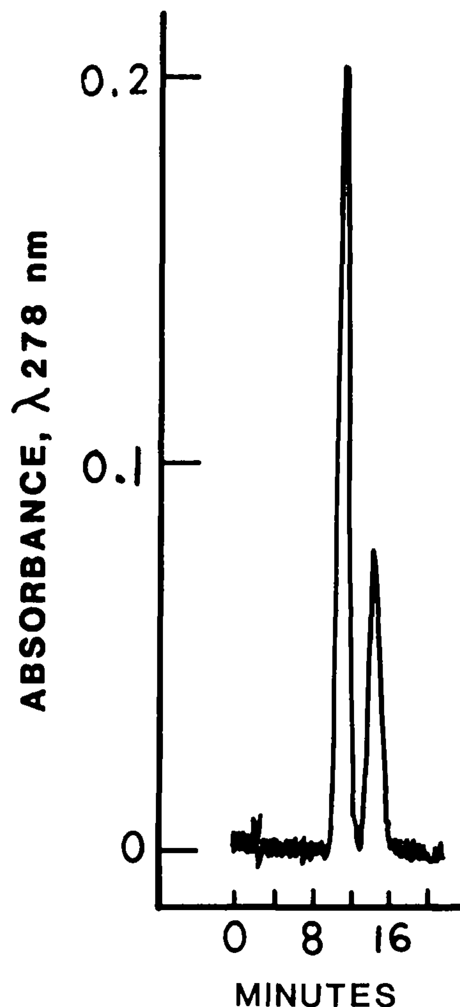


Figure 1—*Paired-ion high-performance liquid chromatogram of commercial sample of I. The major peak corresponds to isomer A (II), and the minor peak corresponds to isomer B (III), which were subsequently isolated from the sample by fractional crystallization.*

With the identity of the two constituents of the commercial sample of I established, it was concluded that the NF reference standard consisted solely of potassium guaiacol-4-sulfonate, inasmuch as its NMR and IR (see below) spectra were identical to those for II, and the retention time of its single HPLC peak coincided with that for II (see above).

The obvious difference in the compositions of the commercial sample of I and the NF reference standard raises some concern regarding the reliability of the identification tests and assay procedure described in NF XIII (17), since the methodologies involved rely on direct comparison of the drug with the reference standard. It was important, therefore, that the accuracy of these methods be reevaluated.

[16] Performed by Galbraith Laboratories, Inc., Knoxville, Tenn.
[17] Zeromatic IV pH meter; Beckman Instruments.

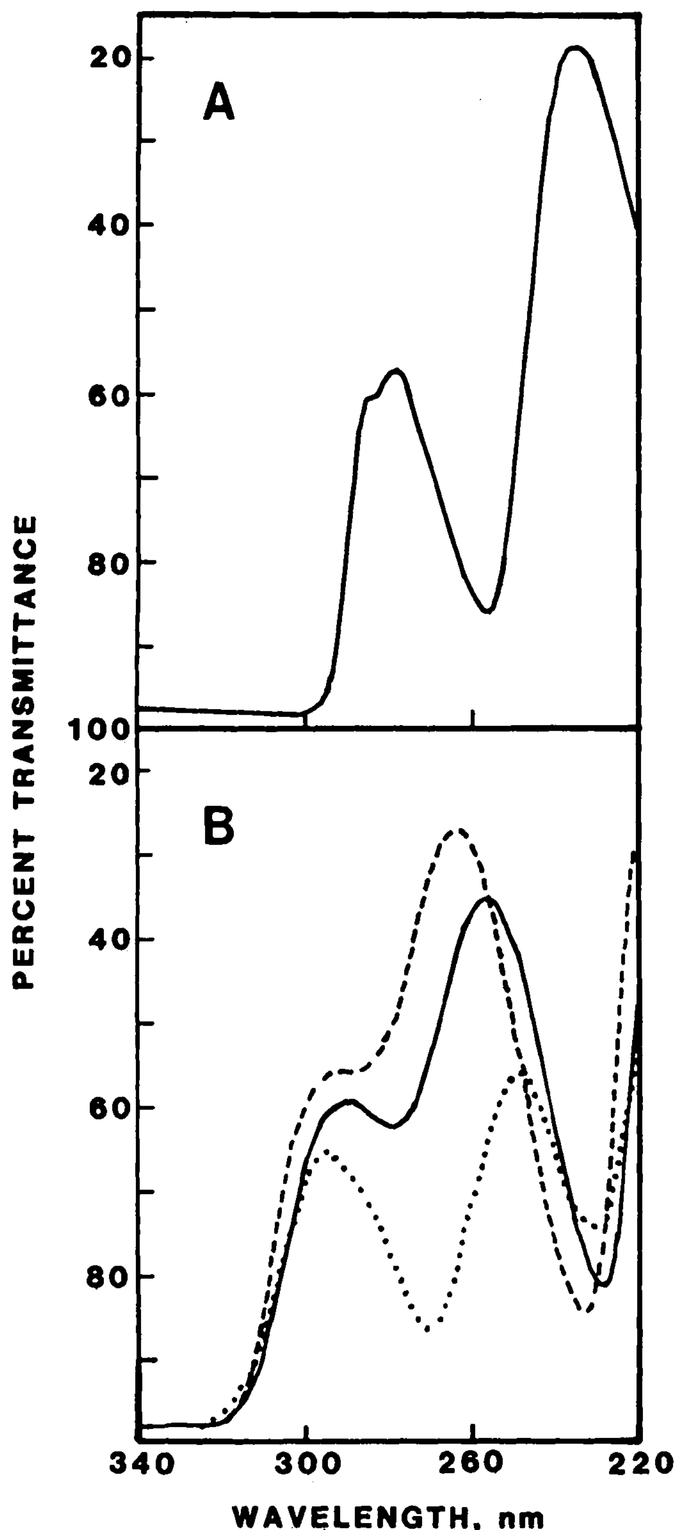


Figure 2—*(A) The identical UV spectra (1-cm path length cells) of 0.02-mg/mL solutions of II, III, and a commercial preparation of I in water, 0.1 M HCl, and pH 7.0 phosphate buffer. (B) UV spectra (1-cm path length cells) of 0.01-mg/mL solutions of II (- - - -), III (· · · ·), and a commercial preparation of I (——) in 0.1 M NaOH.*

Evaluation of the NF IR Absorption Identification Test for I (17)—As expected, the IR spectra of KBr dispersions of pure II and the NF reference standard I proved to be superimposable, confirming the chemical identity of the standard as the single pure compound. However, the IR spectrum for the commercial preparation of I differed markedly from that of the reference standard in the fingerprint region (especially around 1100–1300 cm^{-1}) due to the contribution of absorption bands from its second constituent, III. Therefore, it must be concluded that comparison of the IR spectra of commercial and reference standard samples of I cannot be used as a rigorous criterion for the reliable identification of unknown samples of the drug.

Evaluation of the NF UV Absorption Identification Test and Assay Procedure for I (17)—Dabrowski and Patel (18), although not acknowledging that they were comparing different materials, claimed that their reference standard and commercial preparations of I exhibited the same UV behavior in aqueous solution at pH ≤ 7, and on this basis proposed an assay for the drug which was adopted by the NF. This methodology is only valid provided that isomers II and III have identical UV absorption characteristics.

Indeed, the UV spectra of II, III, and the commercial sample of I in aqueous solution all appear to be identical at pH ≤ 7 (Fig. 2A), with absorption maxima at 236 and 279 nm, as previously reported for the commercial preparation of I (18, 21, 22). However, in 0.1 M NaOH, the spectra for the two pure isomers differ substantially (Fig. 2B), with II exhibiting absorption at λ_{max} of 264 and 293 nm and III exhibiting absorption at λ_{max} of 249 and 296 nm. The spectrum for the commercial preparation of I (Fig. 2B) is clearly a composite of the contributions from its two isomeric constituents, with absorption maxima at 257 and 290 nm. Based on these results, it can be concluded that despite the difference in their compositions, comparison of the UV spectra of the commercial mixture and NF reference standard in pH 7.0 buffer solution (but not in alkaline solution), as specified in NF XIII, is a valid identification test. However, the same comparison for quantitation of I is only reliable provided that the absorptivities of the two isomeric constituents, II and III, are identical at the wavelength of analysis (279 nm).

Determination of the molar absorptivities of the two isomers from the slopes of the respective Beer's law plots ($r > 0.999$, with intercepts passing through the origin verified by t test) yielded values of ϵ of $3.07 \times 10^3 \pm 0.03 \times 10^3$ and $2.97 \times 10^3 \pm 0.01 \times 10^3$ L $mol^{-1} \cdot cm^{-1}$ for II and III, respectively, at λ_{max} of 279 nm in pH 7.0 buffer solution. Statistical comparison of these values by a two-tailed t test revealed that their difference is not significant at the $p = 0.05$ level but is significant at the $p = 0.1$ level. Thus, if a 95% or higher probability level of significance is acceptable, the two absorptivities can be considered to be not different, and therefore, the UV spectrometric quantitation of the commercial drug by direct comparison with the single-constituent NF reference standard appears to be justified under the specified conditions.

Spectrometric Estimation of the pK_a Values of the Isomers of I—The aforementioned differences in the UV spectra of the acidic and basic forms of II and III could be taken advantage of to determine their pK_a values (phenol group). The values obtained were 8.74 ± 0.05 for II and 9.16 ± 0.04 for III, which compare with a pK_a of 9.98 for guaiacol (23). The greater acidity of the 4-sulfonate *versus* the 5-sulfonate derivative is consistent with the relative acidities of *p*- and *m*-methylsulfonylphenol, which have pK_a values of 7.83 and 8.40, respectively (23).

CONCLUSIONS

The determination of the composition of the commercial preparation of I to be a mixture of the guaiacol-4- and -5-sulfonate salts, whereas the NF reference standard of I has been shown to be only pure potassium guaiacol-4-sulfonate, has raised questions concerning the reliability of direct comparison of the two materials for the identification and assay of the drug as specified in NF XIII. However, based on the present work, it appears that as long as the UV assay procedure is carried out in neutral or acidic solutions of the drug, valid quantitative results can be obtained. In contrast, comparison of the IR spectra of commercial *versus* reference standard I is not strictly reliable as an identification test for the drug, because significant differences in the fingerprint regions which distinguish the two pure isomers from each other also cause the commercial sample to present a composite spectrum which is different from that of the pure, single-component reference standard.

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Synthesis of Bridged Catechol–Homocysteine Derivatives as Potential Inhibitors of Catechol *O*-Methyltransferase

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Abstract □ Catechol derivatives, covalently joined to homocysteine by sulfide or sulfonium linkages, were synthesized as potential catechol *O*-methyltransferase multisubstrate inhibitors which might bridge the enzymatic binding sites for the catechol substrate and the amino acid portion of the methyl donor *S*-adenosylmethionine. These compounds were found to be less effective inhibitors than the product inhibitor *S*-adenosylhomocysteine.

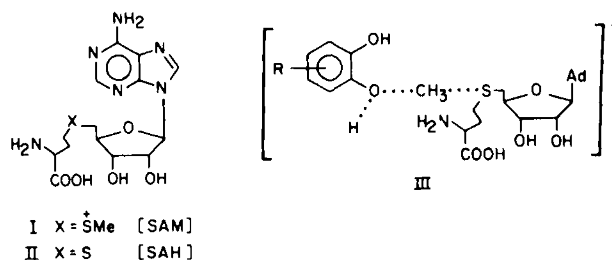
Keyphrases □ Catechol homocysteine derivatives—bridged, synthesis, inhibition of catechol *O*-methyltransferase □ Catechol *O*-methyltransferase—potential inhibitors, bridged catechol–homocysteine derivatives

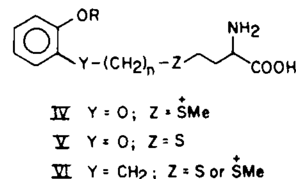
Catechol *O*-methyltransferase (COMT; EC 2.1.1.6) catalyzes methyl transfer from *S*-adenosyl-L-methionine (SAM, I) to catechol substrates and plays an important role in the inactivation of catecholamines (1, 2). COMT inhibitors offer opportunities for control of catecholamine levels and have potential for treatment of disorders thought to arise from catecholamine deficiencies (3). For example, by decreasing the formation of *O*-methylated metabolites of levodopa and dopamine, COMT inhibitors may enhance the effectiveness of levodopa therapy for Parkinsonism (3–5).

One approach to design of inhibitors of SAM-dependent methylases has focused on analogues of SAM or the product inhibitor *S*-adenosyl-L-homocysteine (SAH, II) (6–12). However, analogues of SAM and SAH may not be highly specific inhibitors for a single methylase since many methyltransferases utilize SAM as the methyl donor and are also subject to potent product inhibition by SAH (1). Although inhibitor specificity for COMT might be more readily achieved with catechol analogues, a number of potent *in vitro* inhibitors of this type are limited in clinical potential by poor *in vivo* activity and/or by unacceptable toxicity (3).

An intriguing alternative approach to COMT inhibitor design emanates from consideration of the proposed mechanism for methyl transfer by COMT. The mechanism appears to involve an S_N2-like nucleophilic attack by the catechol on the methyl group of SAM (13, 14) with a transition state which may be depicted as shown in III. This suggests that compounds such as IV and V, in which a catechol is covalently joined to homocysteine by sulfide or sulfonium linkages, might bridge the enzymatic binding sites for the catechol and for the amino acid portion of SAM and thus might function as multisubstrate (15), or rudimentary transition-state (16, 17), inhibitors of COMT.

The direct transition state analogue IV (n = 1) would not be expected to be sufficiently stable to permit study in an aqueous environment. In an earlier study (18), this problem was circumvented by isosteric replacement of one of the catechol oxygen atoms by a methylene group (*cf.* VI, n = 1). The adducts thus obtained were stable, but were not potent COMT





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