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Identification of Some Interferences in the Analysis of Clorazepate

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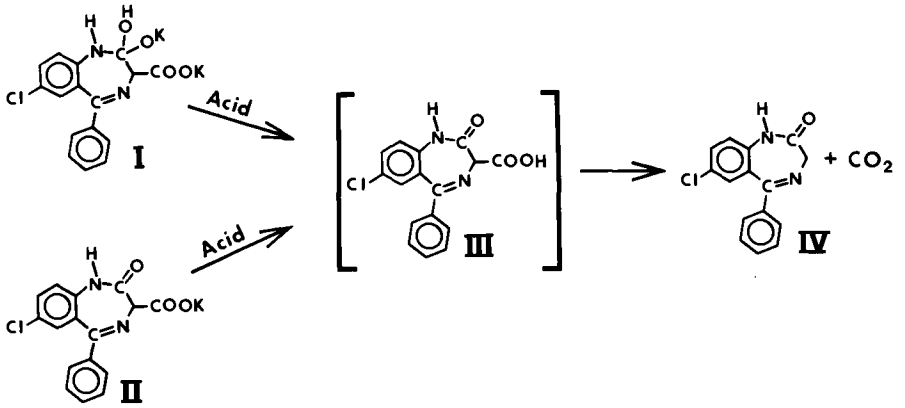
ABSTRACT: Clorazepate presents several problems in identification. In addition to rapid acid decarboxylation to *N*-desmethyldiazepam, a noncontrolled substance often confused with clorazepate, extracts of the pharmaceutical forms (Tranxene® and Azene® capsules) contain substances that interfere with isolation of intact and unaltered clorazepate. These substances have been identified and have been found to be quite dependent on both capsule type and, especially, on capsule age. The cause of the conversion of dipotassium clorazepate to the monopotassium salt, following solution, has also been identified. An infrared analysis method, which removes all of the interferences, is presented.

KEYWORDS: toxicology, clorazepate, spectroscopic analysis

Clorazepate, a Schedule IV benzodiazepine, occurs as the dipotassium (I) and monopotassium (II) salts in the pharmaceutical products Tranxene® (Abbott Laboratories) and Azene® (Endo Laboratories), respectively. The free acid form of these salts (III) is very unstable[2] and readily decarboxylates. Acid extracts of clorazepate therefore yield the decarbox-



Structures of dipotassium clorazepate (I), monopotassium clorazepate (II), the unstable free acid of clorazepate (III), and N-*desmethyldiazepam (IV).*

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[2]The -NCOCH(COOH)- moiety of III is responsible for this instability and gives a structure that is analogous to malonic acid and acetoacetic acid. These are known to decarboxylate readily as exemplified in the malonic ester and acetoacetic ester synthesis routes for carboxylic acids and ketones, respectively.

ylation product, *N*-desmethyldiazepam, (IV, nordiazepam). As this latter compound is not presently controlled under the Uniform Controlled Substances Act and at least two crime laboratories have recently reported receiving white powders [*1*] or counterfeit tablets [*2*] containing this substance, it is imperative that the forensic chemist be able to distinguish clearly between clorazepate and *N*-desmethyldiazepam and correctly identify clorazepate.

An extraction procedure for Tranxene using concentrated ammonium hydroxide solution has been described (1977), with infrared or mass spectral identification [*3*]. Infrared spectra characteristic of clorazepate are not obtainable with this method, however. An X-ray diffraction report (1980) on clorazepate has noted that this failure arises from decarboxylation in water or aqueous NH_4OH [*4*]; the authors mentioned that excipients may contribute to this problem, although the nature of this interference was not specified. On the basis of the solubility of clorazepate in various solvents [*5*] and the problems encountered with water or aqueous NH_4OH, the authors concluded that there is no solvent system suitable for isolating clorazepate, and thus an infrared identification of the mixed form of this drug is not possible.

Unfortunately, most other conventional analytical methods are also not applicable for clorazepate. The high polarity and low volatility of clorazepate salts hamper a gas chromatographic identification; higher column temperatures promote thermal decarboxylation and hense gas chromatography/mass spectrometry (GC/MS) yields, at best,[3] only a spectrum of *N*-desmethyldiazepam. On top of this, the mass spectra of clorazepate and *N*-desmethyldiazepam are essentially identical with the exception of a carbon dioxide ion fragment at $m/e = 44$ for clorazepate; there are no observable parent peaks for clorazepate [*3–5*]. The usual methods of overcoming this problem, namely, various types of esterifications of the carboxylate group, are made more difficult by the ease of decarboxylation under acid or heated reaction conditions. There are currently no thin-layer chromatography (TLC) solvent systems[5] capable of separating clorazepate and *N*-desmethyldiazepam, nor any suitable microcrystalline tests. The ultraviolet spectra [*7*] of clorazepate and *N*-desmethyldiazepam are very similar, and neither compound gives definitive results with color screening tests. High-pressure liquid chromatography (HPLC) has been used successfully on clorazepate [*7,8*], although some decarboxylation occurs. Powder X-ray diffraction has been used successfully for identifying clorazepate intact in the mixed pharmaceutical capsules [*4*], but Tranxene tablets were not tested and many laboratories are not equipped with such an instrument.

In the course of an investigation into a suitable identification procedure for clorazepate, the interferences, which previously prevented an infrared analysis of this compound from extracts of diluted forms, were noted and subsequently identified. Since there has been con-

[3]It has been observed that aqueous solutions of dipotassium and monopotassium clorazepate and methanol solution of *N*-desmethyldiazepam give the same peak shapes and retention times by gas chromatography (OV-17, 250°C isothermal, Ref. *6* and Footnote 4); mixtures of clorazepate with *N*-desmethyldiazepam in methanol-water were not separated. Interestingly, methanol solutions of clorazepate gave very weak or no observable peaks (this does not appear to be due to clorazepate solubility differences in methanol and water). Since III is a key intermediate in decarboxylation, this last observation probably reflects a kinetics effect; that is, methanol boils away before significant amounts of III are formed while water, because of its greater acidity and heat of vaporization, can act as an efficient proton donor. In any case, it is very unlikely that clorazepate and *N*-desmethyldiazepam would have similar retention times considering their very pronounced differences in polarity.

[4]G. L. Ashmore, Coordinator, Northwest Association of Forensic Scientists Proficiency, Solid Dosage #22, Wyoming Department of Agriculture, private communication, Dec. 1980.

[5]A commercially available drug detection system, Toxi-lab® (Analytical Systems, Inc.) and Ref *6* list several TLC methods for identification of clorazepate. None of these are capable of separating clorazepate from *N*-desmethyldiazepam. Furthermore, in both extraction procedures nonpolar solvents (in which clorazepate is mostly insoluble) are used, hence any *N*-desmethyldiazepam present (or formed) would be preferentially sampled. We have learned[6] that abnormally large amounts of clorazepate standard were necessary to detect spots using one of these procedures.

[6]W. C. Romel, private communication, Analytical Systems, Inc., at 33rd Annual Meeting of the American Academy of Forensic Sciences, Los Angeles, CA, 17–20 Feb. 1981.

siderable interest recently in the problems[7] of identifying clorazepate [3,4], these results are described here. In addition, some identification procedures developed from this work, including an infrared method, several TLC solvent systems, and an ultraviolet screening method, are presented.

Experimental Procedure

Standards of dipotassium clorazepate, monopotassium clorazepate, and *N*-desmethyldiazepam were obtained from Abbott Laboratories. Tranxene capsules and tablets (15, 7.5, and 3.75 mg), Tranxene SD® time release tablets (22.5 and 11.25 mg), and Azene capsules (13, 6.5, and 3.25 mg) were purchased locally. The following were also used: potassium hydroxide, potassium carbonate (anhydrous), potassium bicarbonate, and sulfuric acid, Mallinckradt; potassium carbonate (hydrated), MCB; talc, potassium chloride, ammonium chloride, ammonium bicarbonate, hexanes, sodium carbonate (anhydrous), sodium bicarbonate, and "Baker Instra-Analyzed"® cyclohexane, J. T. Baker; dimethylformamide dimethylacetal "Methyl-8"®, Pierce; palmitic and stearic acids, methyl palmitate, methyl stearate, and methyl oleate, Poly Science Corp.; potassium bromide (infrared grade), 2-amino-5-chlorobenzophenone, and glycine, Sigma; and cellulose (cotton), Absorbent Cotton Co. Potassium stearate was prepared by mixing methanol solutions of equimolar stearic acid and potassium hydroxide, and collecting and drying the resulting residue that floated to the top of this solution.

Infrared spectra were recorded on either a Perkin-Elmer 467 or a Beckman 252 CMX. Frequency measurements, computer subtraction of spectra, and other spectral calculations were performed using the latter instrument. All infrared spectra were taken of samples pressed into 13-mm KBr pellets.

Gas chromatography was performed on either a Hewlett-Packard 5840A or a Hewlett-Packard 5710A. The 5840A was equipped with a flame ionization detector, a slightly modified HP-18835B capillary inlet system, and a Hewlett-Packard 50-m by 0.2-mm inner diameter fused silica wall coated open tubular (WCOT) column with a methyl silicone liquid phase. The carrier gas was hydrogen at 2.0-kg/cm^2 constant pressure, and the temperature program was 180°C for 2 min, and then 8°C per minute to 240 or to 272°C, depending on the sample. The 5710A was equipped with two treated glass columns (3% OV-17 on Gas Chrom Q and 3% Dexsil on Anakrom Q) and flame ionization detectors. A temperature program of 120°C for 2 min to 280°C at 16° per minute was used for most work.

Ultraviolet spectra were recorded on a Beckman Acta V and a Hewlett-Packard 8450A spectrophotometer equipped with a Hewlett-Packard 7225A plotter. Peak wavelengths and first derivative spectra were obtained on the latter instrument.

Elemental analyses (for elements with atomic numbers greater than that of neon) were performed on a Kevex 0700-7000 X-ray fluorescence spectrometer using a rhodium primary target. Secondary targets of gadolinium, tin, silver, germanium, iron, and titanium were also used. All samples were placed over a taut 0.00038-cm (0.00015-in.) Mylar® polyester film for analysis.

Results and Discussion

Infrared spectra of dipotassium clorazepate, monopotassium clorazepate, and *N*-desmethyldiazepam are shown in Figs. 1*a*, 1*b*, and 2*a*, respectively. Infrared spectra of the

[7] In a recent regional proficiency program[4] 21 white powder samples consisting of 4 mg of dipotassium clorazepate and 300 mg of mannitol were sent to 15 participating member laboratories (May 1980). Nine replies (an unusually low number) were received in 60 days, with four of these correctly identifying clorazepate.

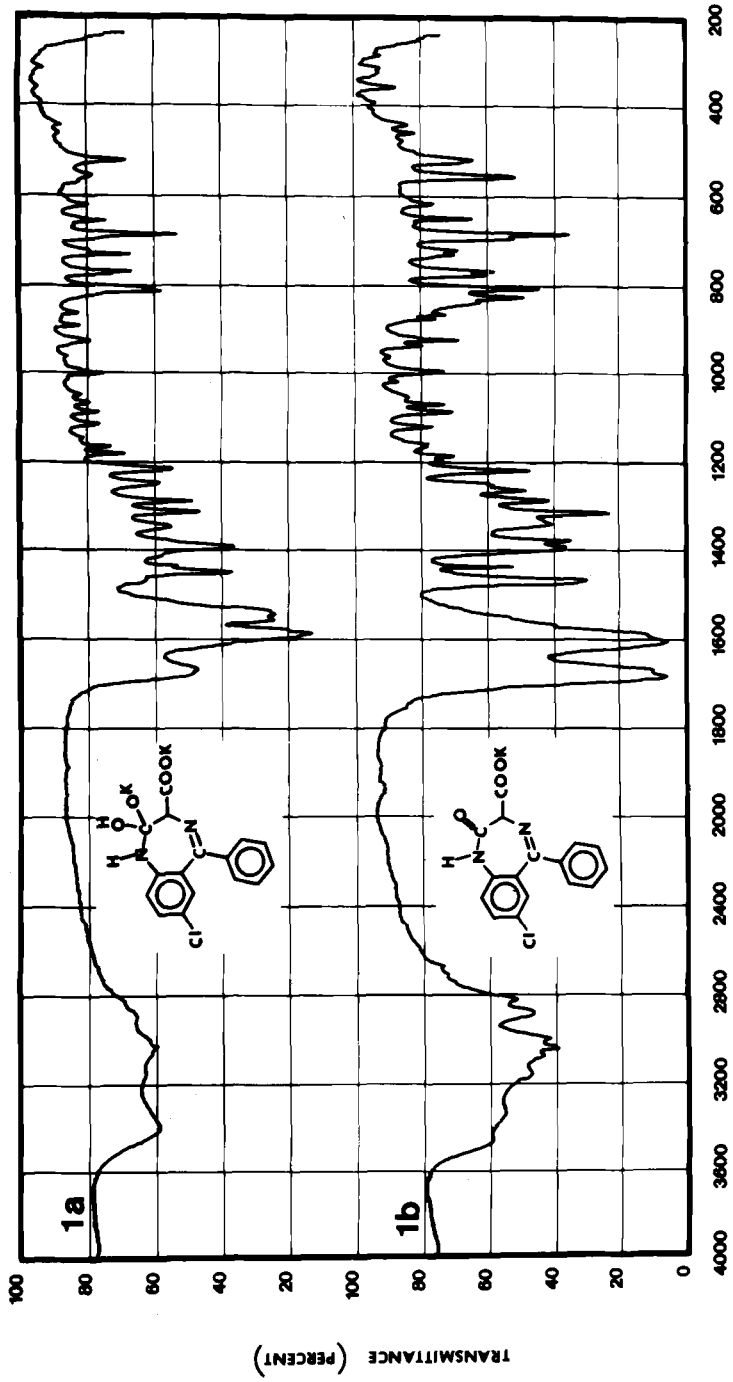


FIG. 1—(a) *Dipotassium clorazepate and* (b) *monopotassium clorazepate.*

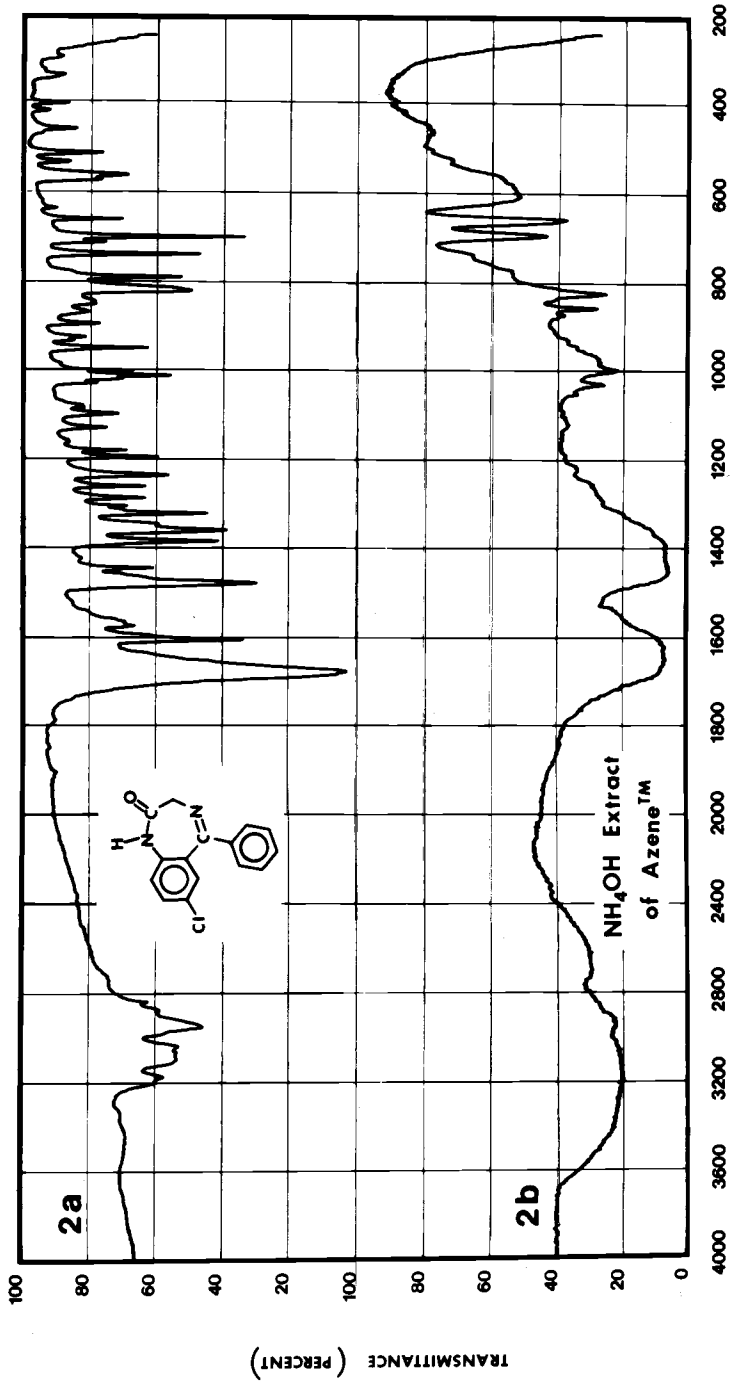


FIG. 2—(a) N-*desmethyldiazepam and* (b) *evaporated residue of a concentrated* NH_4OH *extract of the powder from a 3.25-mg Azene capsule.*

evaporated residues resulting from concentrated aqueous NH_4OH [3] extracts of Azene and Tranxene (capsules) are shown in Figs. 2*b* and 3*a*, respectively; certain Tranxene capsules gave results similar to those of Azene, and some of the absorptions in the Azene extract spectra (Fig. 2*b*) changed quite dramatically in relative intensities in the first few hours following formation of such pellets. In any case, it may be seen that these extract spectra bear little similarity to those of the standards. Analogous extracts using water alone and methanol (which we have found also to be a suitable solvent for clorazepate) were examined, with results for Azene shown in Figs. 7*a* and 6*b*, respectively; the observed spectral differences using NH_4OH, water, and methanol (Figs. 2*b*, 7*a*, and 6*b*) indicate that several components may be responsible. Extracts of Tranxene (of the type giving NH_4OH extracts as in Fig. 3*a*) with water or methanol, on the other hand, gave results identical to Fig. 3*a*. Extracting these Tranxenes with other organic solvents, while not yielding clorazepate spectra, often resulted in fairly simple infrared spectra with absorptions (at 3667, 1018, 668, 466, 454, and 392 cm^{-1}) also observed in the NH_4OH, aqueous, or methanol extracts (Fig. 3*a*). At least two components are thus indicated as interfering in these capsules also.

From infrared spectra of the capsule contents of the various dosages of Tranxene and Azene, two types of material have been identified as the primary (infrared absorbing) filler in these products. Spectra of the contents of two types of 15-mg Tranxene are shown in Fig 3. All of the older Tranxene capsules that were tested (at all three dosage levels) gave spectra similar to Fig. 3*c*; above, Fig. 3*b*, is shown the spectrum of talc, which may be seen to be a principal constituent in older Tranxene capsules. Since talc is a layer-type silicate, it is probably suspended, rather than solvated, in the extraction procedure. Consistent with this, the talc absorptions in the Tranxene extracts (Fig. 3*a*) are absent, or much weaker, when such extracts are filtered.

For the newer Tranxene capsules that were examined, spectra of the contents gave results similar to that of Fig 3*d*. All of the Azene capsules examined gave similar results (for example, Figs. 4*a* and 4*b*). From a comparison of Figs. 3*d*, 4*a*, and 4*b*, with Fig. 3*b*, it is clear that talc is also a major ingredient in these capsules. In addition, a water-insoluble, particulate, cellulose-like component is also present. Figure 4*d* shows the spectrum of the residue from an Azene capsule powder that was washed several times successively with water, methanol, and chloroform; above, Fig. 4*c*, is the spectrum of cellulose (cotton). Except for the absorptions of talc (Fig. 3*b*) in the former (indicated by arrows), the two spectra are essentially identifical. This cellulose filler, however, does not readily form a suspension and is not directly responsible for the remaining interfering absorptions.

In order to determine whether these remaining absorptions can be attributed to properties of clorazepate alone, the clorazepate standards were dissolved in methanol (or water) and the evaporated residues examined. The infrared spectrum of the monopotassium salt did not change significantly (Fig. 13*a*), except for peak broadening. As Fig. 5*a* indicates, however, the dipotassium salt has converted to the monopotassium salt, an observation that was previously noted for NH_4OH solutions [3], but not explained.

Since the dipotassium salt is quite alkaline, the behavior of solutions of hydroxide ion upon evaporation is noteworthy. Following evaporation of potassium hydroxide solutions, for example, $KHCO_3$, K_2CO_3 or both result (see below, Reactions 1 and 2). $KHCO_3$ is the

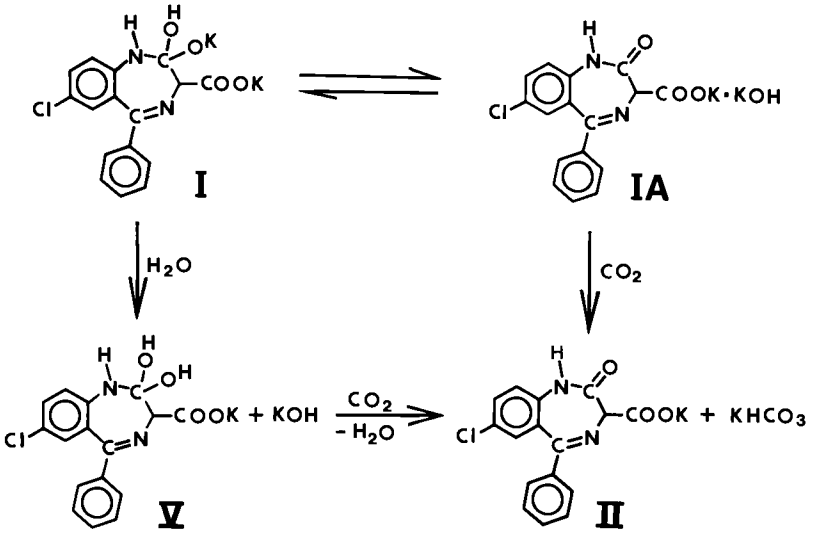
$$OH^- + CO_2 \text{ (solution)} \rightarrow HCO_3^- \quad (1)$$

$$OH^- + HCO_3^- \rightarrow CO_3^- + H_2O \quad (2)$$

primary product when low initial concentrations of potassium hydroxide are used. These reactions occur (with carbon dioxide vapor) even for solid potassium hydroxide.

A number of tautomeric forms [9] are possible for dipotassium clorazepate, two of which are shown below as I and IA. In solution, the potassium hydroxide of IA may combine with

dissolved atmospheric CO_2; complete titration (via Reaction 1 above) and evaporation of solvent would then yield equimolar monopotassium clorazepate and $KHCO_3$. In addition, I may form a *geminal* diol intermediate (V) in aqueous solution which dehydrates to give the same results.



Two of the tautomeric forms of dipotassium clorazepate (I and IA) and their conversion to monopotassium clorazepate (II) in solutions exposed to atmospheric carbon dioxide.

Consistent with this, the conversion occurs in an atmosphere of CO_2 alone, and not in the absence of CO_2. In addition, absorptions of $KHCO_3$ (Fig. 6*a*) may be seen in the dipotassium salt conversion product spectrum (Fig. 5*a*) with relative intensities comparable to those observed in the spectrum (Fig. 5*b*) of an equimolar mixture[8] of monopotassium clorazepate and $KHCO_3$. Further supporting this, a subtraction of the spectrum of the monopotassium salt (residue of an aqueous solution of monopotassium clorazepate) from the spectrum of the conversion product (residue of an aqueous solution of dipotassium clorazepate) yielded a spectrum of $KHCO_3$.

For solvent extracts of Tranxene (which contains the dipotassium salt), one would thus expect the appearance of a small amount of $KHCO_3$. Instead, almost all of the extracts of both Tranxene (Fig 3*a*) and Azene (Figs. 2*b* and 6*b*) show very prominent $KHCO_3$ absorptions (Fig. 6*a*). In particular, all of the extracts of older Tranxene and the methanol extracts of Azene (and newer type Tranxenes) are almost exclusively $KHCO_3$ (compare Figs. 3*a* and 6*b* to 6*a*); clearly there is much more $KHCO_3$ present than can be accounted for by the above conversion alone. Spectra of the capsule contents of all of the older Tranxenes examined (those having only talc as the primary filler) along with some older Azenes, do in fact show distinct absorptions of $KHCO_3$ (for example, compare Figs. 3*c* and 4*a* to 6*a*).

In addition, absorptions of hydrated K_2CO_3 are observed in some residues of water and aqueous NH_4OH extracts. Figure 7*a* for example, shows the result of an aqueous extract of an Azene (3.25-mg) capsule; Fig. 7*b* is a spectrum of the residue resulting from evaporation

[8]In comparing this spectrum to that of the monopotassium clorazepate standard itself (Fig. 1*b*), it may be seen that instead of the appearance of new peaks in the prepared mixture, already existing peaks appear to be enhanced in relative intensity. $KHCO_3$, however, does not appear to be a significant impurity in monopotassium clorazepate, as indicated by the quantitative elemental analysis (discussed later). Despite this, it is worth mentioning that $KHCO_3$ would not be a totally unexpected impurity. The original synthesis of monopotassium clorazepate consisted of an aqueous solution neutralization of dipotassium clorazepate with monopotassium phosphate; some $KHCO_3$ (along K_2HPO_4) would also be expected to be produced.

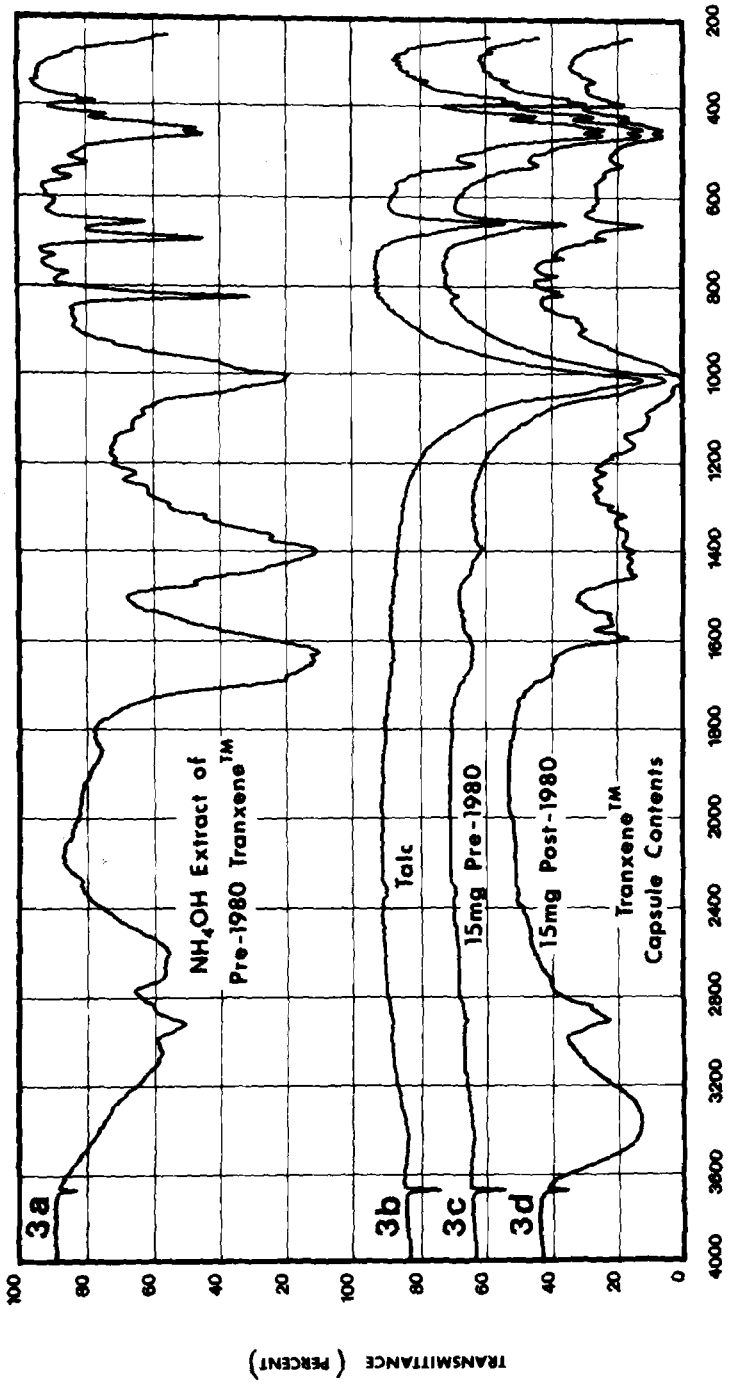


FIG. 3—(a) *Evaporated residue of a concentrated NH_4OH extract of the powder from a pre-1980 (talc only) 7.5-mg Tranxene capsule,* (b) *talc,* (c) *powder from a pre-1980 (talc only) 15-mg Tranxene capsule, and* (d) *powder from a post-1980 (talc + cellulose) 15-mg Tranxene capsule.*

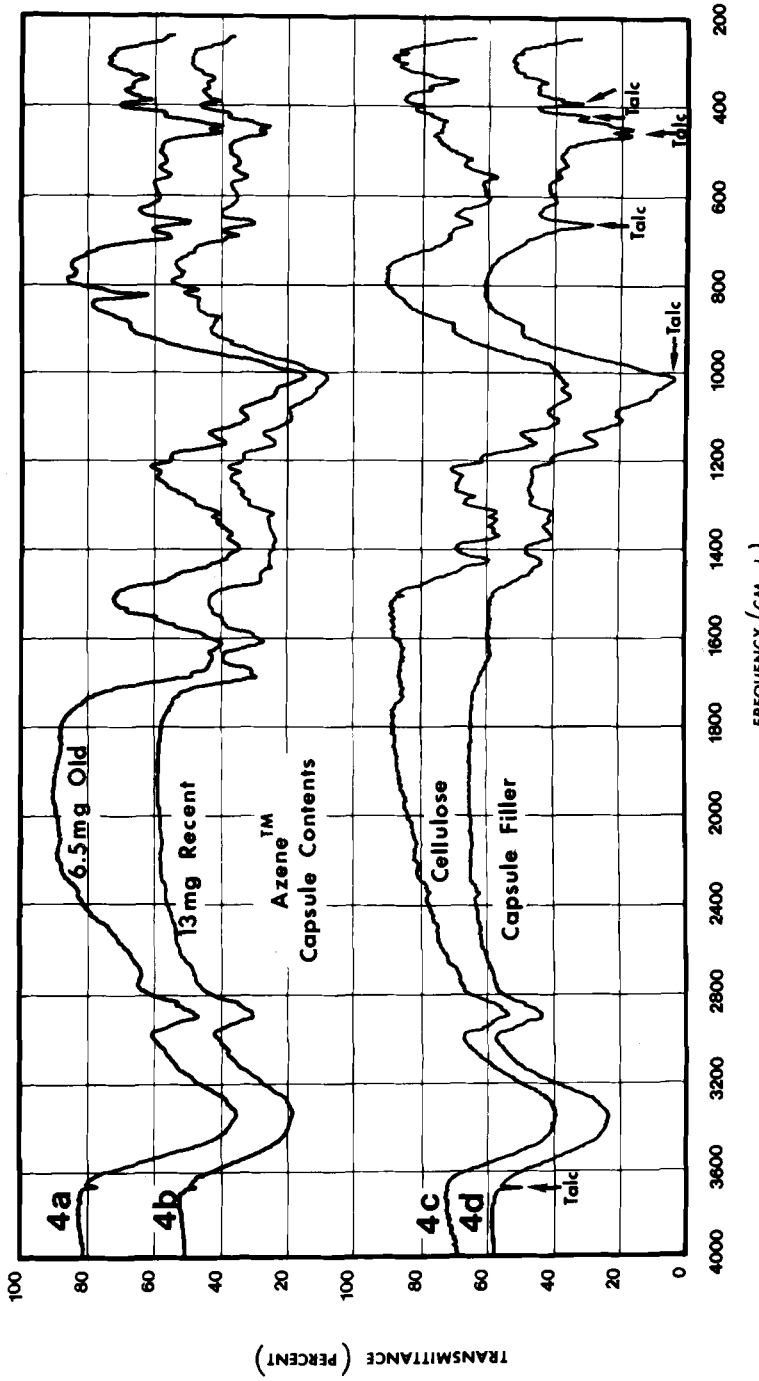


FIG. 4—(a) *Powder from a relatively old 6.5-mg Azene capsule,* (b) *powder from a recent 13-mg Azene capsule,* (c) *cotton (cellulose), and* (d) *composite powder from various dosages of Azene and post-1980 Tranxene capsules after several washes with water, methanol, and chloroform; powder dried overnight at 60°C. Talc absorptions are indicated by arrows.*

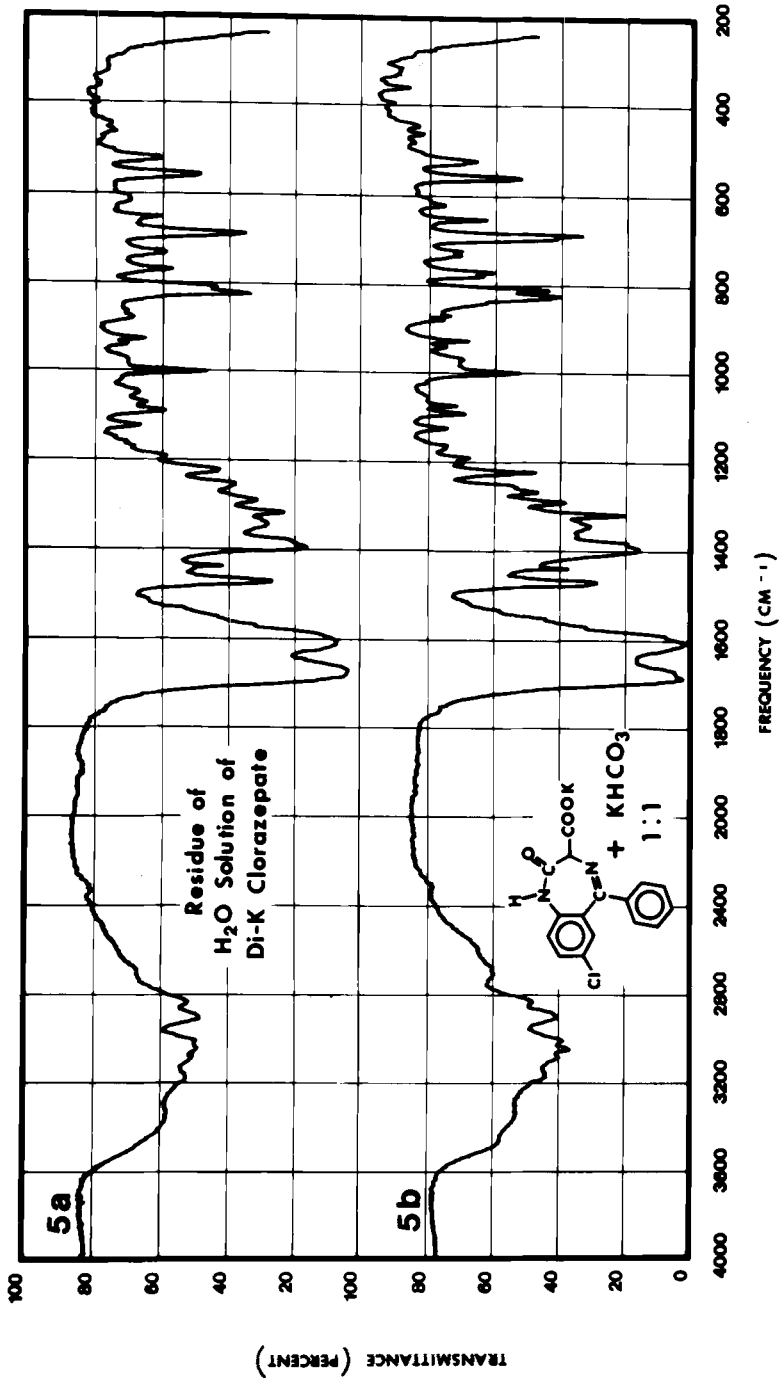


FIG. 5—(a) *Evaporated residue of an aqueous solution of dispotassium clorazepate and* (b) *equimolar mixture of monopotassium clorazepate and* $KHCO_3$.

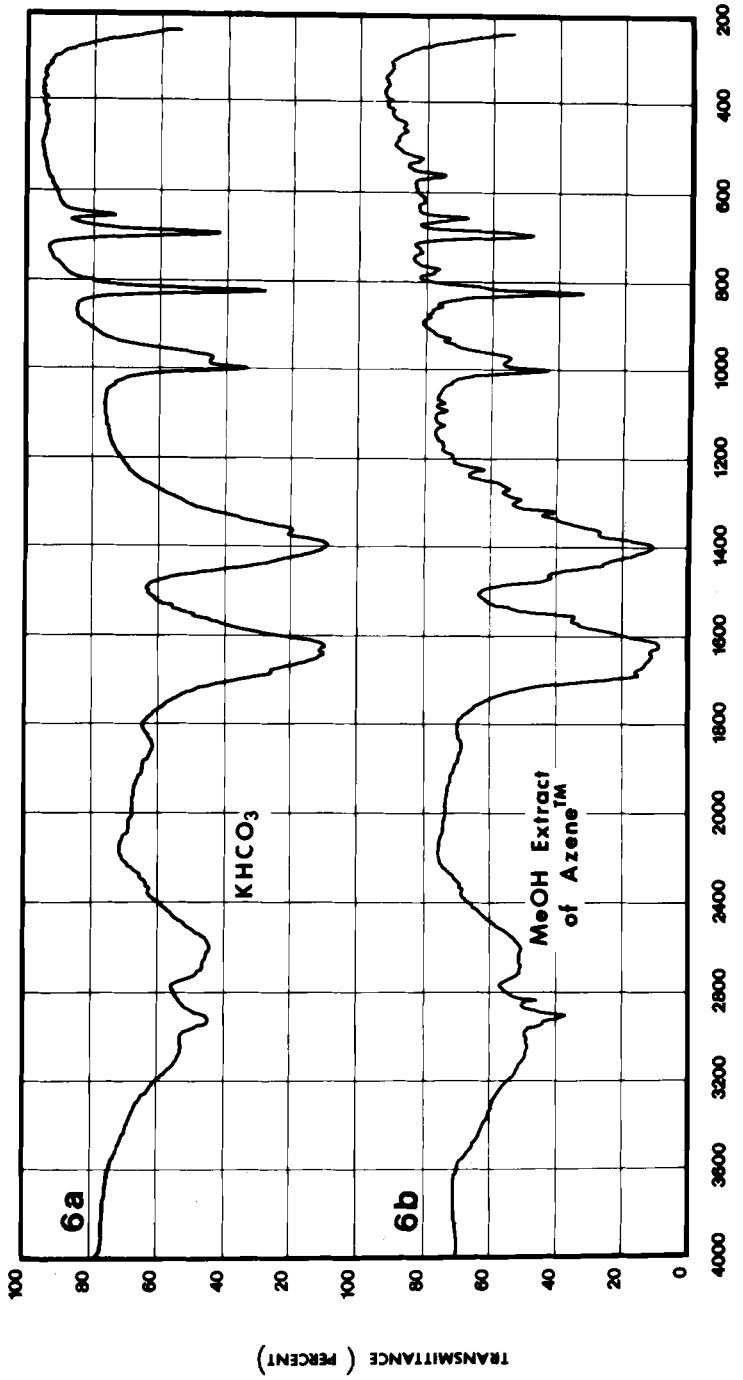


FIG. 6—(a) *$KHCO_3$ and* (b) *evaporated residue of a methanol extract of the powder from a 6.5-mg Azene capsule.*

of an aqueous solution of K_2CO_3. The latter spectrum is primarily that of $K_2CO_3 \cdot H_2O$ and $K_2CO_3 \cdot 1.5\ H_2O$ (Fig. 8*b*, Ref *10*) along with a considerable amount of trapped lattice water, as evidenced by the broad absorptions at 1650 and 600 cm^{-1} [*11*]. The K_2CO_3 residue spectrum also shows the pronounced low frequency absorption intensity changes observed in many of the Tranxene and Azene extract spectra, which probably reflect hydation changes or annealing of the various K_2CO_3 hydrated forms or both. Weak absorptions consistent with K_2CO_3 are observed in spectra of the capsule contents of Azene and newer type Tranxene.

It is worth mentioning that differences exist in the infrared spectra of the carbonates (and especially in the bicarbonates) of potassium and sodium. Other alkali/alkaline earth metal[9] salts can also be ruled out.

While investigating possible microcrystalline tests for clorazepate, it was observed that aqueous extracts of Azene and Tranxene (newer types) gave immediate, thick, white precipitates with silver nitrate.[10] Microscopic examinations of the powder from these capsules showed, amongst other particles, numerous transparent isotropic cubes; these were confirmed to be potassium chloride from their optical dispersion properties.

The presence of potassium chloride, which itself does not absorb in the infrared region above 300 cm^{-1}, accounts for an observation that could not be explained by the presence of K_2CO_3 or $KHCO_3$ alone, namely, the increased amount of $KHCO_3$ in aqueous NH_4OH extracts of Azene or newer type Tranxene compared to aqueous extracts. Evaporation of potassium chloride dissolved in NH_4OH solution yields a mixture of NH_4Cl and $KHCO_3$, the latter resulting from Reaction 1 above. (NH_4Cl has absorptions at 3120 and 1402 cm^{-1}, both of which are obscured by the strong K_2CO_3 [hydrated] or $KHCO_3$ absorptions; NH_4HCO_3 does not form in appreciable amounts under these conditions.)

That intact di- and mono-potassium clorozepate are present in their respective capsule powders may be seen from Figs. 3*d* (content of a newer type 15-mg Tranxene) and 4*b* (contents of a recent 13-mg Azene) wherein the characteristic doublets of di- and mono-potassium clorazepate at 1600 and 1550 cm^{-1} and 1690 and 1610 cm^{-1}, respectively, are clearly observed (these absorptions are usually obscured in older capsules because of the increased amount of $KHCO_3$—see for example Fig. 4*a*). What is desired for extraction is a clorazepate-dissolving solvent that effectively excludes $KHCO_3$, K_2CO_3, and potassium chloride. A chloroform-menthanol 3:1 mixture (CM 3:1) has been found to work adequately for this purpose.

In spite of the insolubility of $KHCO_3$ and K_2CO_3 in this solvent, varying amounts of $KHCO_3$ or K_2CO_3 or both are sometimes observed in CM 3:1 extracts. This suggests that potassium hydroxide (which is quite soluble in CM 3:1) might be responsible (via Reaction 1). To verify this, powder from a very recent Tranxene was divided into two portions, one of which was extracted immediately with CM 3:1 and filtered. The remaining portion was placed in a desiccator containing CO_2 and water for 1 h, then extracted. The untreated sample gave a spectrum having very pronounced $KHCO_3$ absorptions, while the one treated with CO_2 had very little $KHCO_3$.

A further indication of the presence of KOH was obtained from examination of changes in the composition of the capsule powder itself. A KBr pellet of the powder from a very recent capsule that had been kept in a desiccator was prepared in an atmosphere of dry nitrogen, and its spectrum recorded. The remainder of this powder was exposed to a CO_2 atmosphere as above, then stored in a desiccator again for several days before recording its spectrum.

[9]Elemental analyses of the powder from Azene and Tranxene capsules indicate the presence of magnesium (talc), silicon (talc), chlorine, potassium, and iron. The iron levels, relative to silicon, are essentially identical to those observed (as an impurity) in U.S.P. grade talc. The newer type Tranxene (with the cellulose filler) and Azene contain more chlorine and potassium than the older Tranxene. These capsules also contain a small amount of bromine, the levels of which are comparable to those observed (as an impurity) in reagent grade potassium chloride (discussed later).

[10]Aqueous solutions of monopotassium clorazepate give turbid solutions forming more slowly than the AgCl precipitate. This could be due to silver clorazepate. As this turbid product did not readily precipitate with centrifugation and was not retained by most filters, its identity was not pursued.

The spectra, with and without treatment, are very similar to those of Fig. 4*a* and 4*b*, respectively (which are actually spectra of the contents of a relatively old 6.5-mg Azene and a newer 13-mg Azene). Subtraction of one spectrum from the other revealed that the following changes occurred after CO_2 exposure: (1) a significant *loss* of a relatively sharp absorption near 3450-cm^{-1}, (2) loss of absorptions corresponding to those of anhydrous K_2CO_3, and (3) a pronounced increase in the absorptions of $KHCO_3$. The loss of the 3450-cm^{-1} absorption is quite significant in view of the fact that exposure to water vapor would normally be expected to increase hydroxyl absorptions as a result of hydration of the various components present. The frequency and relative sharpness of this absorption are consistent with an hydroxide anion [*11*].

Only capsules of very recent manufacture seem to have appreciable amounts of hydroxide present; a similar correlation appears to occur for capsules giving mainly K_2CO_3 for residues of aqueous extracts. This suggests that hydroxide and K_2CO_3 are slowly neutralized by atmospheric CO_2, in the presence of moisture, to $KHCO_3$. This is also supported by the relative intensities of the $KHCO_3$-K_2CO_3 absorptions observed in spectra of the powder from capsules of various ages (for example, Fig. 4*a* and 4*b*). Since the presence of hydroxide alone will eventually produce K_2CO_3 and $KHCO_3$ (and also may produce these substances during extraction), whether K_2CO_3 and $KHCO_3$ are actually original capsule ingredients is uncertain. In any case, the older capsules of Azene and Tranxene contain mostly $KHCO_3$, which is extracted by aqueous NH_4OH, water, or methanol. For the newer capsules, the extraction of K_2CO_3 with water and only $KHCO_3$ with methanol is consistent with the solubilities of these two salts.

For the older type Tranxene (having only talc as the primary filler) potassium hydroxide has not been a problem. However, some filtered CM 3:1 extracts of these capsules, especially those which have been verified as being quite old, give spectra similar to Fig. 9*a*. Several weak absorptions besides those of monopotassium clorazepate (Figs. 1*b* and 13*a*) and talc (Fig. 3*b*) are observed for these samples, which invariably give pellets having a very distinct yellow color. These extra absorptions are seen more clearly in filtered chloroform extracts (Fig. 9*b*) and may be seen to arise, in part, from *N*-desmethyldiazepam (Fig. 2*a*). Also present is another component, which is preferentially extracted with hexane (Fig. 10*a*). This latter substance, which gives rise to the yellow color, has been confirmed to be 2-amino-5-chlorobenzophenone or ACBP (see Fig. 10*b*). Gas chromatography and ultraviolet spectrometry indicate that *N*-desmethyldiazepam and ACBP are the two main components in the chloroform extracts of these older Tranxene samples.

ACBP has been reported [*5*] as an acid hydrolysis product of *N*-desmethyldiazepam. In addition, we have learned from a private communication[11] that ACBP (Structure VI) may form directly from dipotassium chlorazepate through hydrolysis of its ring-opened tautomer, IB [*9*], as indicated in sketch. All of the older type Tranxene capsules that were examined, of various ages, had more ACBP than *N*-desmethyldiazepam present. This, together with the absence of an appreciable amount of ACBP in the *N*-desmethyldiazepam standard and its presence (albeit at quite low levels as indicated by gas chromatography) in the dipotassium clorazepate standard, suggests that ACBP is formed directly from clorazepate in these older, alkaline environment Tranxene capsules.

Quantitation of the levels of ACBP and *N*-desmethyldiazepam in older Tranxene capsules show no clear correlation with age (as indicated by the expiration dates) or dosage level. Capsules from a bottle having an expiration date of December 1978, for example, had average molar yields (based on 3.75-mg dipotassium clorazepate) of 13% ACBP and 8% *N*-desmethyldiazepam. On the other hand, capsules from some bottles with even older expiration dates had lower yields. While these older Tranxene capsules are no longer on the market, they may still be received in forensic science laboratories for analysis.

[11]Hoffman-LaRoche, Inc., private communication, March 1981.

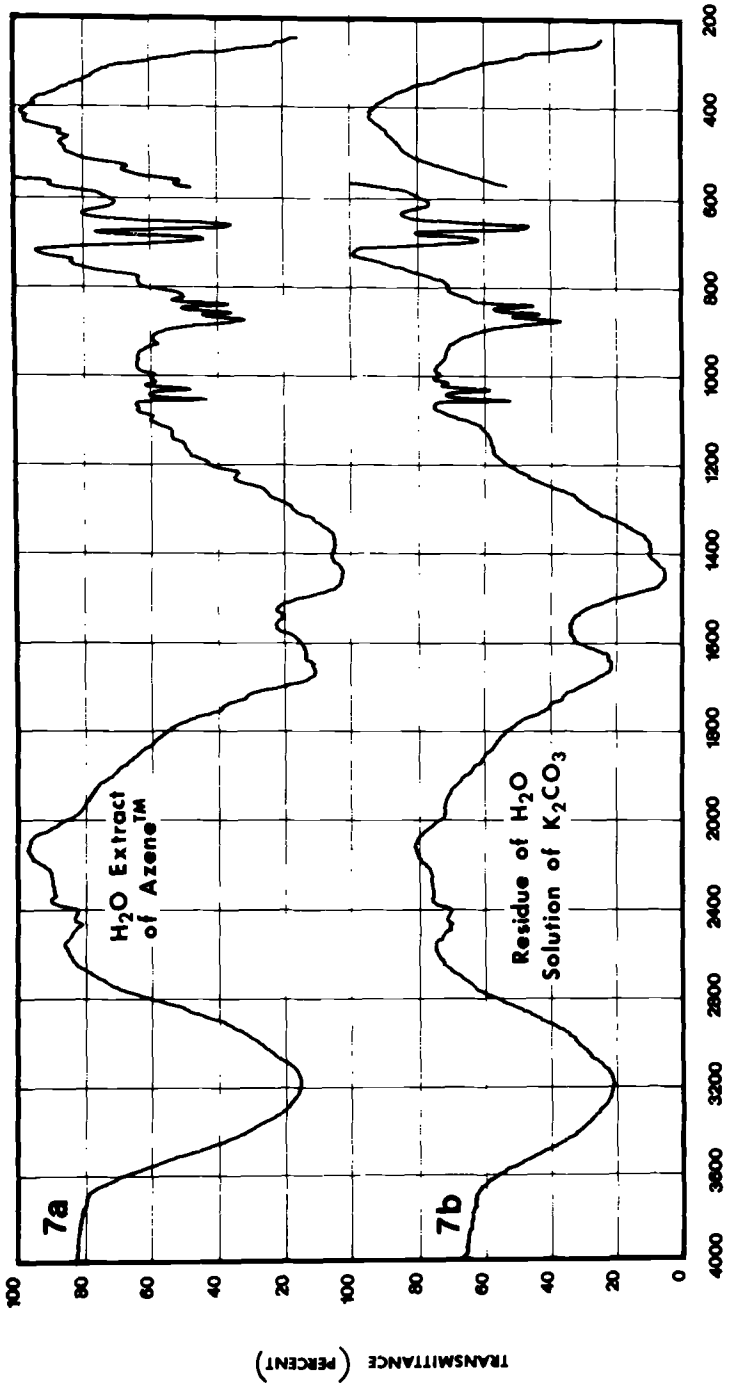


FIG. 7—(a) *Evaporated residue of an aqueous extract of the powder from a fairly recent 3.25-mg Azene capsule, and* (b) *evaporated residue of an aqueous solution of anhydrous* K_2CO_3.

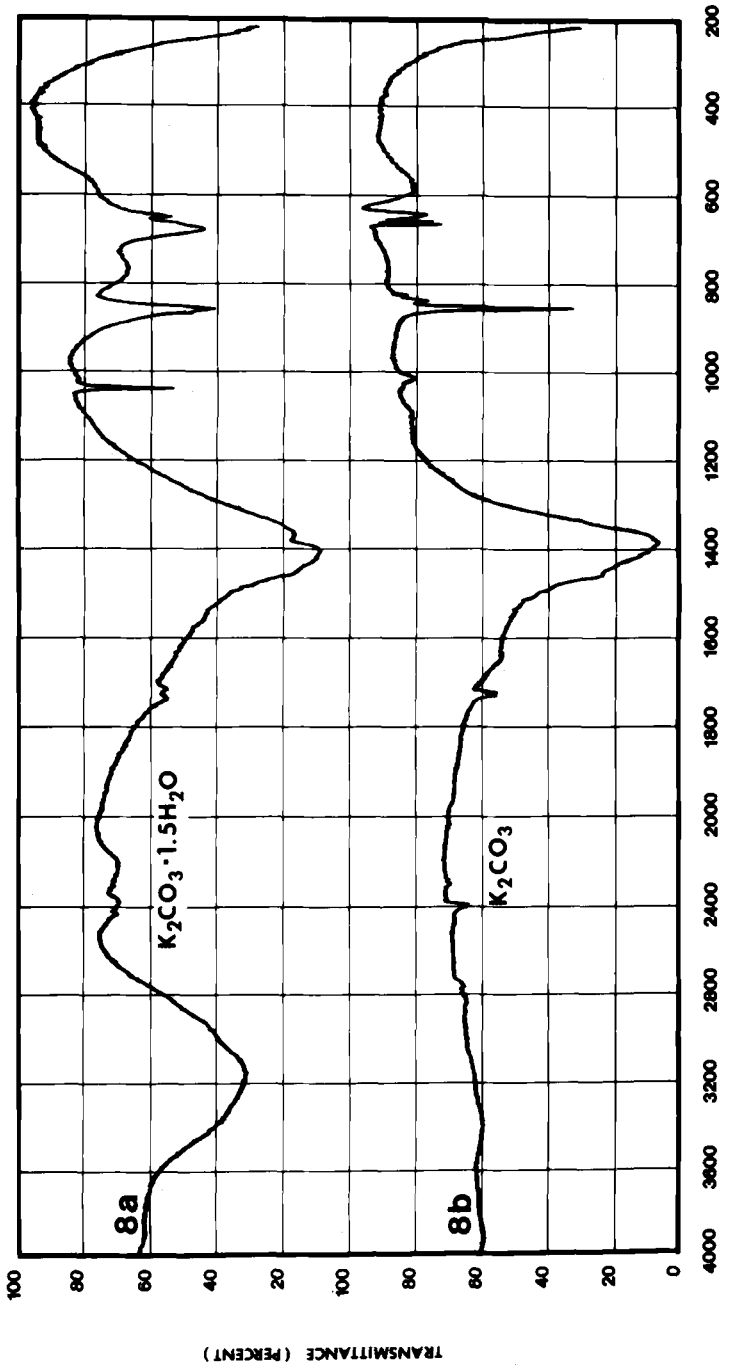


FIG. 8—(a) *$K_2CO_3 \cdot 1.5$ water and* (b) *anhydrous K_2CO_3.*

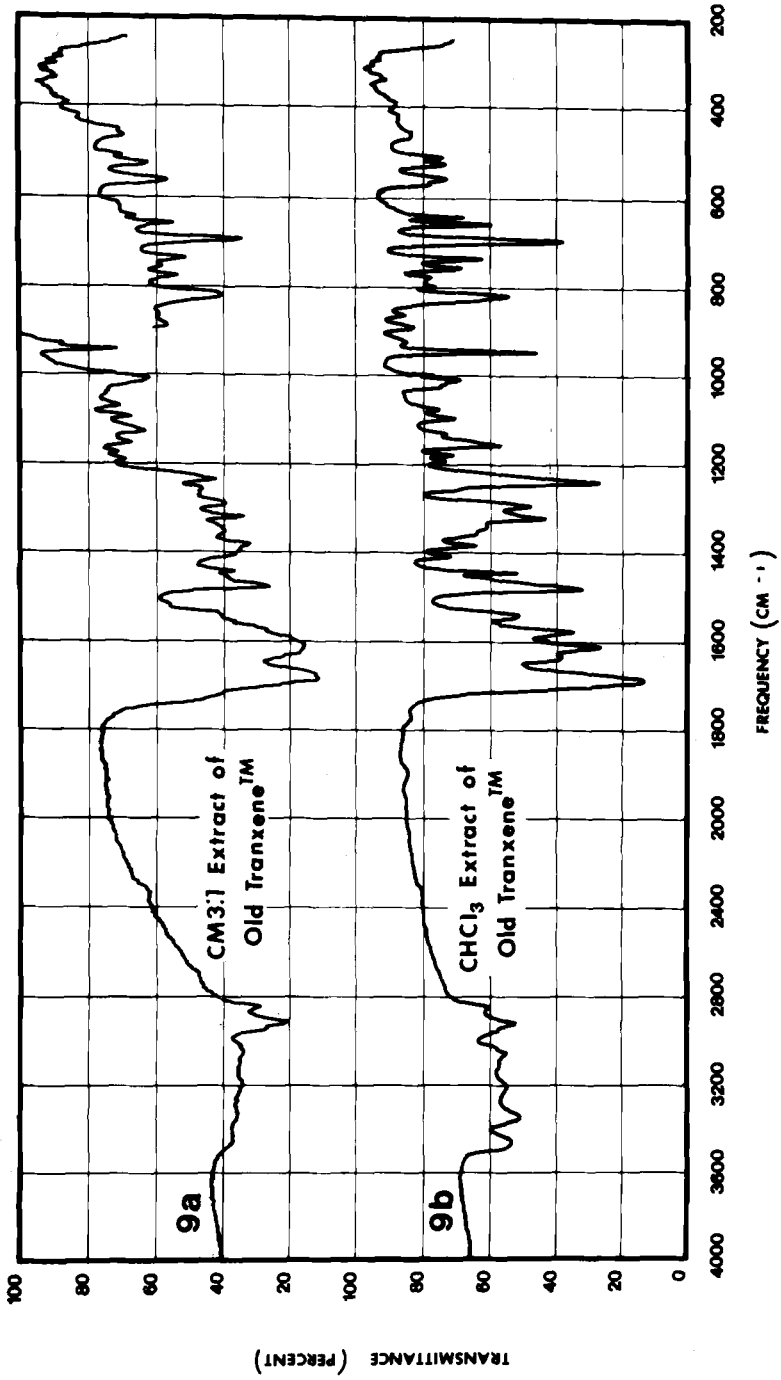


FIG. 9—(a) *Evaporated residue of a filtered CM 3:1 extract of the powder from an old pre-1980 3.75-mg Tranxene capsule bearing the double "a" logo and* (b) *evaporated residue of a filtered chloroform extract of the powder from three old pre-1980 3.75-mg Tranxene capsules all bearing the double "a" logo.*

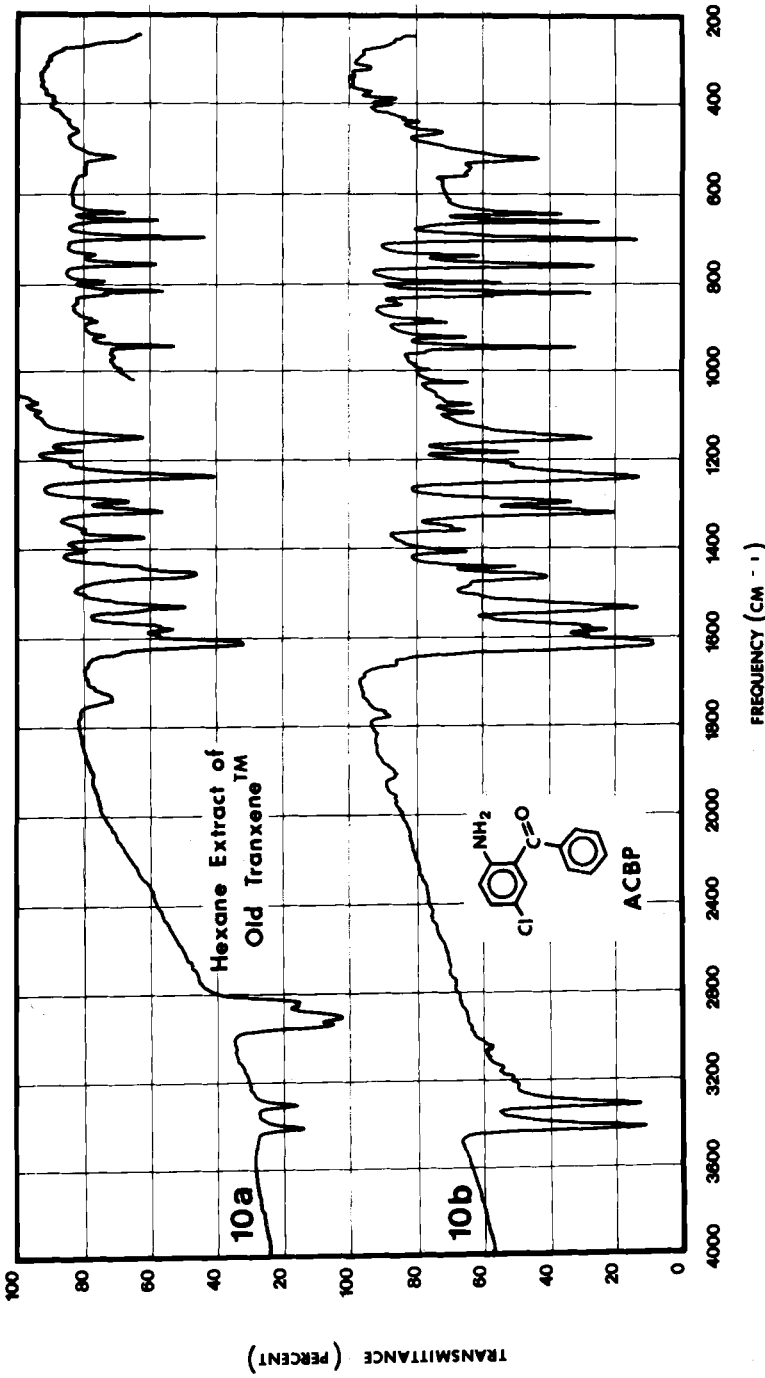
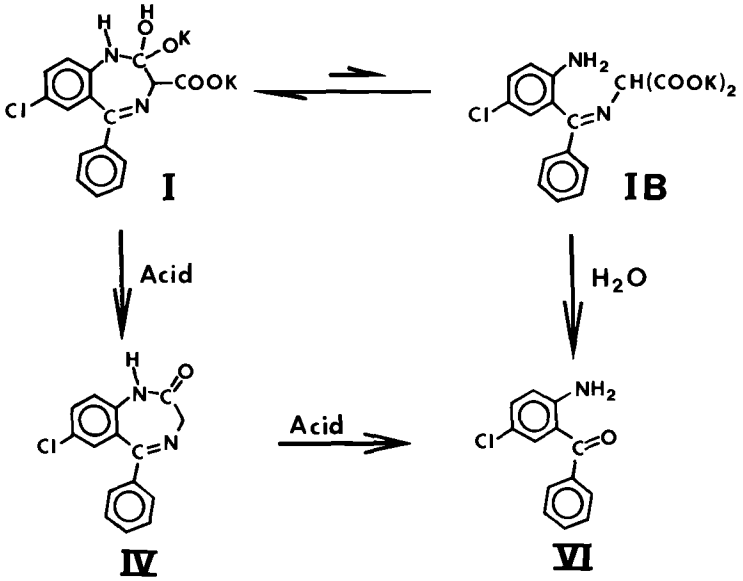


FIG. 10—(a) *Evaporated residue of a filtered hexane extract of the powder from three old pre-1980 3.75-mg Tranxene capsules all bearing the double "a" logo and* (b) *2-amino-5-chlorobenzophenone.*



Two pathways for the hydrolysis of dipotassium clorazepate to ACBP (IV).

The extraneous ACBP and *N*-desmethyldiazepam absorptions in CM 3:1 extract spectra are only observed in some older type Tranxene having talc alone as the primary filler; they are not observed for the newer capsules and Azene, both of which have talc and cellulose filler. For these latter capsules, however, filtered CM 3:1 extracts give spectra similar to Fig. 11*a* (that is, in those cases where the potassium hydroxide has already been neutralized by atmospheric CO_2; otherwise strong absorptions of K_2CO_3 or $KHCO_3$ or both result). Conspicuous nonclorazepate absorptions at 2920, 2853, and 1562 cm^{-1} are observed, which become progressively stronger, relative to the monopotassium clorazepate absorptions, as one extracts lower dosages of Tranxene or Azene.

If these CM 3:1 extract solutions are partitioned into aqueous and chloroform layers (having some methanol in each) with the addition of water, evaporation of the aqueous layer gives spectra of clorazepate having much less of the extraneous absorptions. The chloroform layer gives a spectrum similar to Fig. 12*a*; the intense absorptions at 2920 and 2853 cm^{-1} remain, but a relatively broad absorption is now observed at 1703 cm^{-1}, *not* at 1562 cm^{-1} as seen in the original extract spectrum (Fig. 11*a*). These absorptions are indicative [*12*] of methylene groups (2920, 2853, 1473, 1466, and 723 cm^{-1}) and a dimerized saturated aliphatic acid (1703 and 1300 cm^{-1}); this suggests a relatively long, straight-chained fatty acid structure for the substance(s) isolated in the chloroform layer. Furthermore, the 1562- to 1703-cm^{-1} "shift" is consistent with a carboxylate to free acid conversion (for example, see Figs. 11*b* and 12*b*).

The infrared spectra of the even numbered saturated fatty acids from C14 to C22 [*13*] are all fairly similar to one another (compare for example, Fig. 12*b* and *c*) and to the chloroform layer spectra. Capillary column gas chromatography was thus used for confirmation. For this, hexane extracts (which largely exclude *N*-desmethyldiazepam, see Fig. 12*a*) of sulfuric acid solutions of the capsule contents were examined. Two peaks with retention times corresponding to stearic ($CH_3(CH_2)_{16}COOH$) and palmitic ($CH_3(CH_2COOH$) acids were observed, and these acids were further confirmed by forming and chromatographing their methyl ester derivatives [*14*]. The observation of the stearate and palmitate salts in the original CM 3:1 extract, and their conversion to acids following aqueous partition, indicates that these compounds are probably present as their salts in the original capsule powders.

The basic or hydrophilic nature of most of the substances identified (talc, cellulose, potassium hydroxide, K_2CO_3, $KHCO_3$, potassium chloride, and stearate and palmitate), suggests

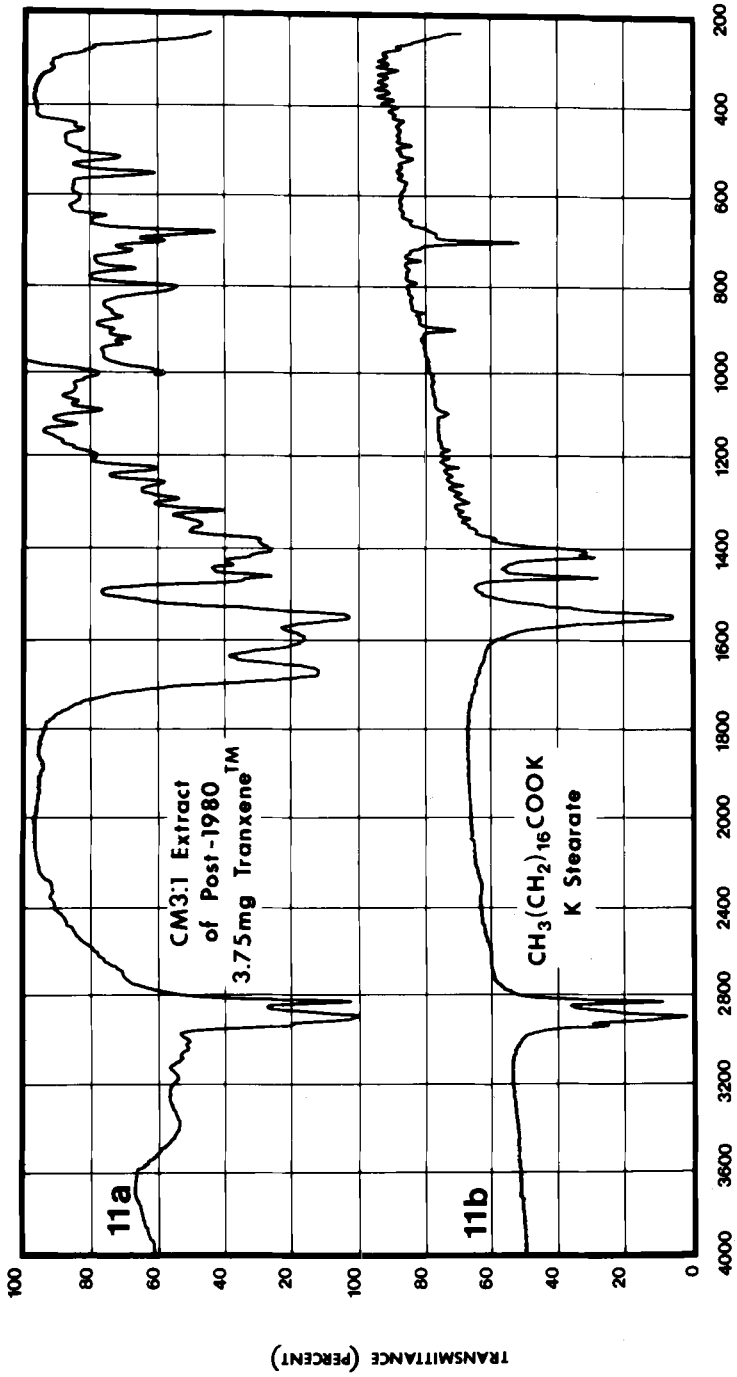


FIG. 11—(a) *Evaporated residue of a CM 3:1 extract of the powder from a post-1980 3.75-mg Tranxene capsule and* (b) *potassium stearate.*

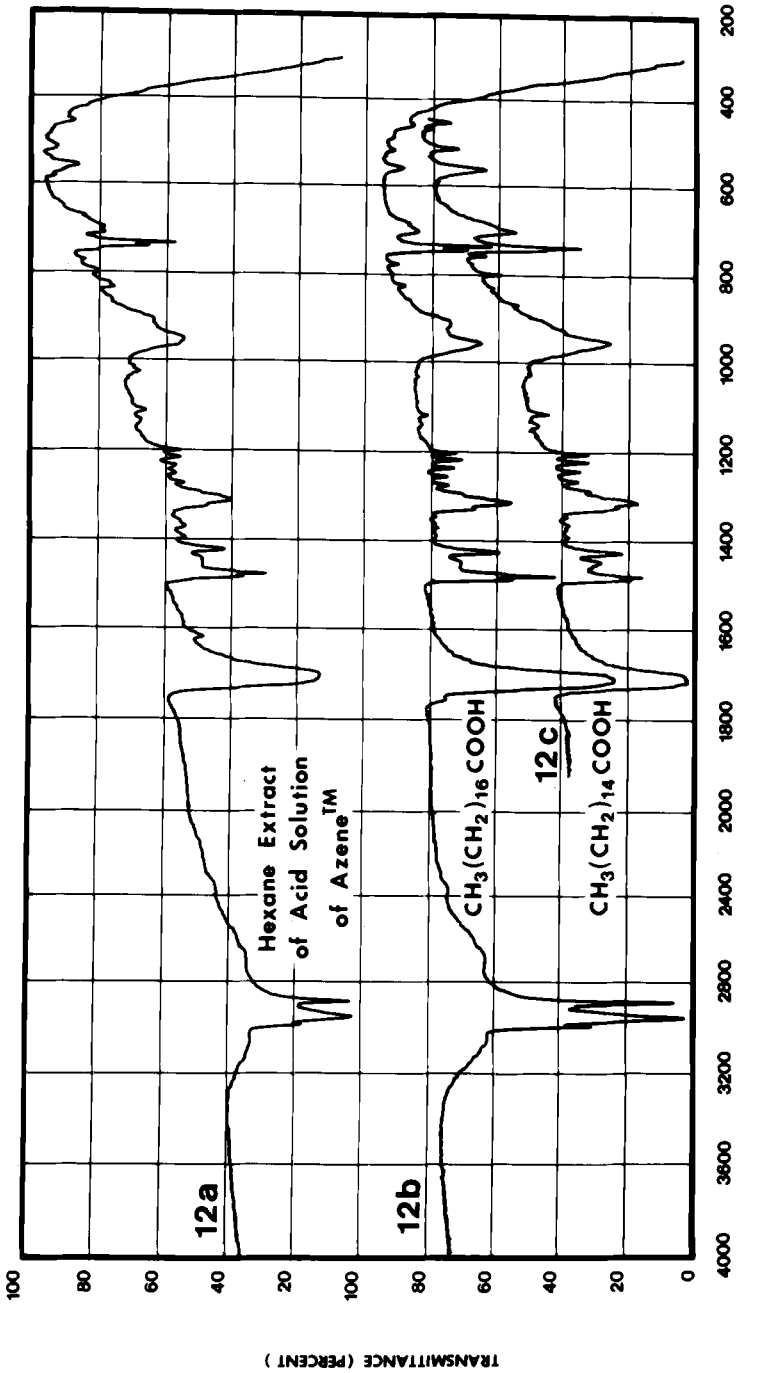


FIG. 12—(a) *Evaporated residue of a hexane extract from a 6N H_2SO_4 solution of the composite powder from various dosages of Azene and post-1980 Tranxene capsules,* (b) *stearic acid, and* (c) *palmitic acid.*

that they may be serving to protect clorazepate from slow decarboxylation and hydrolysis arising from moisture and atmospheric CO_2. Abbott Laboratories (which manufactures both Tranxene and Azene, the latter being marketed by Endo Laboratories), while not disclosing their excipients[12] (except for telling us that microcrystalline cellulose is used), did confirm that their Tranxene filler material was changed in 1980 because of clorazepate stability problems,[14,15] and is now the same as that used in Azene. The oldest talc-only Tranxene capsules (which may have appreciable amounts of *N*-desmethyldiazepam and ACBP) may be identified by their Abbott "a" logo on both the cap and body. Tranxene capsules manufactured after 1975 have identifying letters (CI, CN, or CK depending on dose) along with a single "a" logo.

A summary of the identified capsule ingredients is given in Table 1. The CM 3:1-aqueous partition (above), together with several chloroform washes, not only removes the fatty acid salts but also suspended talc and any ACBP and *N*-desmethyldiazepam that may be present. Pretreatment of the capsule powder with CO_2 and water vapor removes any potassium hydroxide that may be present. Results of this complete procedure (described in detail below)

TABLE 1—*Tranxene and Azene capsule ingredients and extraction scheme.*

Capsule Ingredient	CO_2 Precxtraction Treatmcnt[a]	Extraction Steps		
		CM 3:1	Water Layer	After Washes
Monopotassium clorazepatc (Azenc)	...	soluble	soluble	remains
Dipotassium clorazepate (Tranxcne)	converts to monopotassium salt + $KHCO_3$	converts to monopotassium salt + $KHCO_3$	...	...
Talc	...	forms suspension	partially removed	removed
Potassium hydroxide[b]	neutralized to K_2CO_3 and $KHCO_3$	insoluble as $KHCO_3$ or K_2CO_3	...	...
K_2CO_3	neutralized to $KHCO_3$	insolublc	...	...
$KHCO_3$	...	mostly insolublc	...	...
Stearate salt[c]	...	partially solublc	partially soluble	removed
Palmitatc salt[c]	...	partially soluble	partially soluble	removed
Potassium chloride[c]	...	insoluble	...	...
Cellulose[c]	...	insoluble	...	...
N-desmethyldiazepam[d]	...	soluble	insoluble	...
2-Amino-5-chlorobenzophenone[d]	...	soluble	insoluble	...

[a]Only necessary for some very recently manufactured capsules containing an appreciable amount of potassium hydroxide.

[b]Slowly neutralized (in capsulcs) by atmospheric moisture and CO_2 to K_2CO_3 and eventually to $KHCO_3$. Soluble in CM 3:1 and water if not neutralized; forms K_2CO_3 and $KHCO_3$ upon evaporation.

[c]In Azenc and post-1980 Tranxenc.

[d]Appreciable levels may occur in somc older Tranxene, cspecially in capsules bearing the double "a" Abbott logo.

[12]Complcte listings of the excipients for somc pharmaceutical preparations (including Tranxene and Azenc) are registered with the Food and Drug Administration. We have learned,[13] however, that the Freedom of Information Act has specific proprietary exclusions that prevent public disclosurc of the nonactive ingredients of products so registered. We were informed (in response to an inquiry), however, that potassium hydroxide is permitted by the FDA in some preparations.

[13]U.S. Food and Drug Administration, Washington, DC, private communication, Feb. 1981.

[14]Abbott Laboratories, private communications, May 1980, Dec. 1980, and Jan. 1981.

[15]Endo Laboratories, private communication, May 1980.

for a recently manufactured (potassium hydroxide-containing) 3.75-mg Tranxene are shown in Fig. 13*b*; similar results are obtained for other dosages, Azene, and older capsules, with most of these not requiring a CO_2 treatment. Comparison of this spectrum to that of monopotassium clorazepate (residue of an aqueous solution), Fig. 13*a*, shows that the interfering substances have been removed.

Solvation of clorazepate results in a distinct broadening of most infrared absorptions, as mentioned previously (compare Figs. 13*a* and 1*b*). This may arise from chlorazepate's marked tendency [*9*] to retain solvents (the original preparation of monopotassium clorazepate [*9*], uses a high vacuum drying method), and also, possibly, inability of clorazepate to anneal[16] at ambient temperatures.

Impurities in the Clorazepate Standards

From a decarboxylation of monopotassium clorazepate to *N*-desmethyldiazapam, as monitored through changes in the ultraviolet absorptions, the monopotassium clorazepate standard appeared to contain a significant amount (approximately 5% by weight) of impurity. As $KHCO_3$ was suspected (See Footnote 8), a quantitative elemental analysis was performed[17] with results given in Table 2.

An approximately equimolar amount of water appears to be present, giving a composition of 95.1% for monopotassium clorazepate[18] in good agreement with the decarboxylation results. This residual water is also reflected in the infrared spectrum (Fig. 1*b*, Ref *3*); an hydroxyl absorption at 3400 cm^{-1} is clearly present and is not observed in the spectrum (Fig. 2*a*) of *N*-desmethyldiazepam. This water apparently is bound quite strongly to clorazepate, as high vacuum drying [*9*] does not remove it. This, together with the apparent stoichiometric relation and the fact that many carboxylate salts are hydrated, suggests that monopotassium clorazepate is probably a monohydrate salt.

For the dipotassium clorazepate standard, a distinct growth in the 1690-cm^{-1} carbonyl stretching absorption (Fig. 1*a*) occurs with time; the intensity of this absorption in Fig. 1*a*, for example, was less than half that shown for spectra taken shortly after receiving the sample. Abbott Laboratories,[14] has attributed this growth to decarboxylation arising from atmospheric moisture. Extraction of standard samples (giving prominant carbonyl absorptions) with chloroform, however, fails to remove this absorption, while exposure of dipotassium clorazepate to an atmosphere of CO_2 and moisture for 30 min results in a complete conversion to the monopotassium salt and $KHCO_3$. These observations suggest that a more likely cause of this carbonyl growth is the slow CO_2 neutralization of the dipotassium salt to monopotassium salt via structure IA and Reaction 1. Storage of the dipotassium clorazepate standard in a desiccator containing some potassium hydroxide should retard or prevent this degradation.

Ultraviolet Spectra

The ultraviolet spectra [*1*, *5*, *7*] of clorazepate and *N*-desmethyldiazepam in aqueous alkaline solutions are essentially identical; the latter gives a radically different spectrum in

[16]We have observed that *N*-desmethyldiazepam often appears to form a glass following solution and evaporation of solvent. Infrared spectra of residues taken immediately after pellet formation often give relatively broad absorptions with some overlapping peaks which, after 15 min or so, become sharper and well resolved; concurrently, the scattering background increases. Annealing (prompted, no doubt in part, by the incident infrared radiation) thus has occurred. Since annealing temperature is correlated with melting point (usually beginning at a temperature approximately equal to one half the absolute melting point), clorazepate, being a salt, would be expected to anneal at a higher temperature than *N*-desmethyldiazepam.

[17]Analyzed by Galbraith Laboratories, Inc., Knoxville, TN, Jan. 1981.

[18]The purity of monopotassium clorazepate, as ascertained by Abbott Laboratories, has been reported [*15*] as 94.27%.

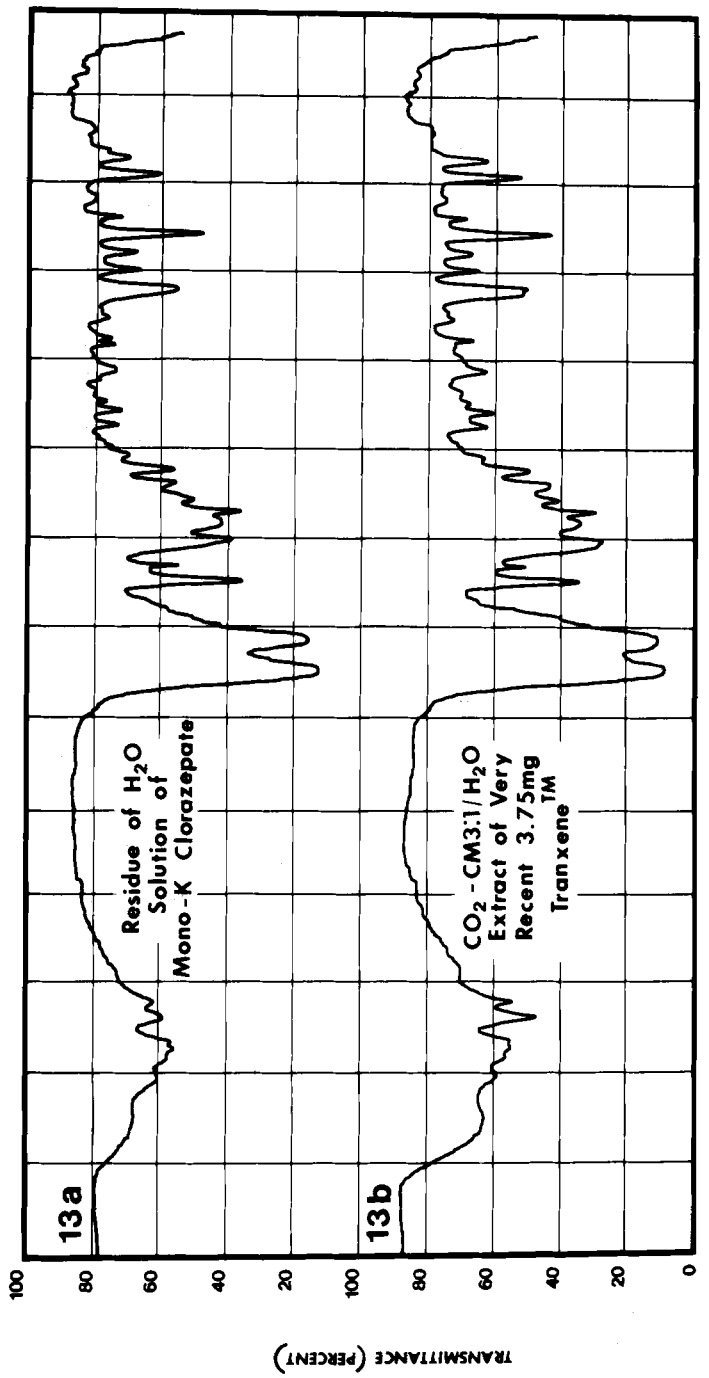


FIG. 13—(a) *Evaporated residue of an aqueous solution of monopotassium clorazepate and* (b) *results using the CO_2 treatment and CM 3:1/water extraction procedure for a potassium hydroxide containing recent 3.75-mg Tranxene capsule.*

TABLE 2—*Elemental analysis results for monopotassium clorazepate.*

Element	% Measured	% Theory	
	Sample from Lot 05-246-CE	Monopotassium Clorazepate	Monopotassium Clorazepate and Water 1:1
Carbon	51.54	54.47	51.83
Hydrogen	3.27	2.86	3.26
Oxygen	17.20	13.60	17.26
Nitrogen	7.47	7.94	7.55
Chlorine	9.74	10.05	9.56
Potassium	10.53	11.08	10.54
Total	99.75	100.00	100.00

acidic solutions while the former decarboxylates. While these spectra cannot easily differentiate the two compounds, the absorbance values of aqueous solutions can give some useful information (in effect being a solubility test). Saturated aqueous solutions of *N*-desmethyldiazepam give absorbance values of less than 2 (1-cm cells) for the 229-nm (strongest) peak. In contrast, saturated clorazepate solutions require considerable dilution before such values are obtained.

It has also been observed that spectra of clorazepate and *N*-desmethyldiazepam in ethanol (and in methanol to a lesser extent) show some differences, which are highlighted in the first derivatives of these absorptions. The clorazepate (both salts) spectrum (Fig. 14*a*) has a very weak absorption at 303 nm not observed for *N*-desmethyldiazepam (Fig. 14*b*). This difference is clearly reflected in the first derivatives (Fig. 14*c* and *d*), wherein clorazepate has a doublet peak at 299 and 317 nm while *N*-desmethyldiazepam has only a single peak at 308 nm. Also seen in the derivative spectra are a distinct peak at 242 nm for clorazepate and a corresponding shoulder for *N*-desmethyldiazepam.

These two distinguishing features of the clorazepate derivative spectrum are seen in most ethanol solutions of Tranxene and Azene capsule contents. They are not observed for derivative spectra of some other *N*-desmethyldiazepam-like benzodiazepines examined (including diazepam, prazepam, lorazepam, and flurazepam), which all give results similar to *N*-desmethyldiazepam. The alkalinity of clorazepate solutions is not the cause of these differences, as the *N*-desmethyldiazepam derivatives are unaffected by changing alkalinity (or the addition of $KHCO_3$). These differentiating features thus undoubtedly reflect the perturbations on the chromophore by the -COOK group of clorazepate (see Structures II and IV.) Interestingly, they are not observed using water or acetronitrile as solvents. They also are not seen in clorazepate samples containing some *N*-desmethyldiazepam; derivative spectra of old Tranxene, for example, resemble those of *N*-desmethyldiazepam, as do those of clorazepate solutions that have been sitting for longer than 30 min. Ultraviolet spectra (and first derivatives) in water and ethanol can thus be used as a rapid screening method for distinguishing the two compounds, with the limitations discussed above.

Identification Methods

Extraction of Clorazepate for Infrared Analysis

Preextraction Treatment for Removal of Hydroxide (Notes 1, 2, and 5)—Spread the powder to be analyzed out on a watchglass. Place in a desiccator containing some hot water and an atmosphere of CO_2 for at least 1 h. Extract as below.

Extraction Steps—

1. Place a quantity of powder from a capsule corresponding to at least 3.75-mg dipotassium clorazepate (or 3.25-mg monopotassium clorazepate) in a test tube(s).

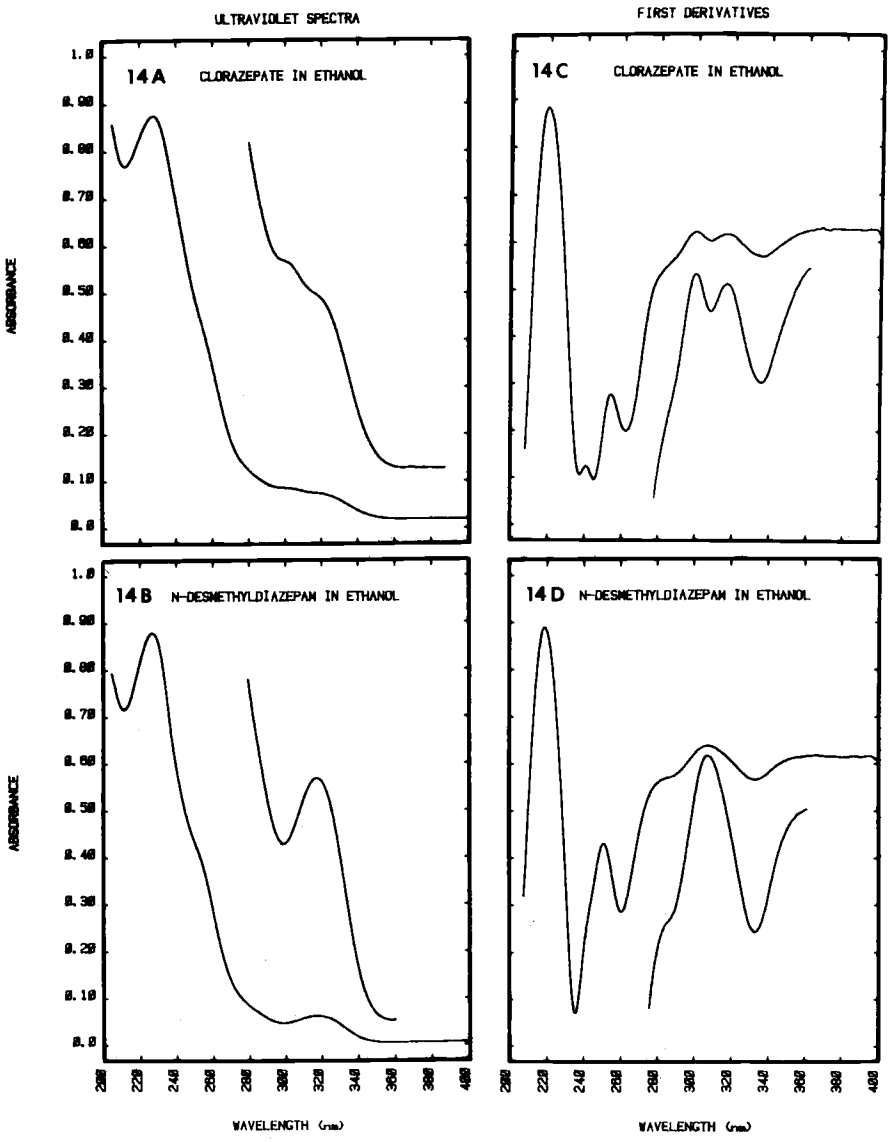


FIG. 14—(a) *Ultraviolet absorption spectrum of monopotassium clorazepate in ethanol,* (b) *ultraviolet absorption spectrum of* N-*desmethyldiazepam in ethanol,* (c) *first derivative of the ultraviolet absorption spectrum of monopotassium clorazepate in ethanol, and* (d) *first derivative of the ultraviolet absorption spectrum of* N-*desmethyldiazepam in ethanol.*

2. Add 12 to 15 mL of CM 3:1 (three parts chloroform and one part methanol by volume) and mix very *vigorously* for at least 2 min.

3. Centrifuge and pipet liquid into a clean test tube. Discard residue (Note 3).

4. Add 1.5 to 2 mL of water, mix thoroughly, and centrifuge.

5. Pipet off the aqueous (top) layer and wash it with chloroform, centrifuging after mixing. Repeat, if necessary, until the white opaque foam-like substance (stearic and palmitic acids) no longer appears in the chloroform layer after centrifuging.

6. Evaporate the aqueous layer to dryness and use all of the resulting residue to form a KBr pellet.

Notes—

1. Although most capsules requiring analysis will probably not contain appreciable amounts of hydroxide, there is no simple a priori method to determine capsule age (other than expiration dates on the capsule bottles, which usually are not available). The pH of aqueous solutions may give some information. The CO_2 exposure has no adverse effect on capsules without hydroxide. Capsules with the double Abbott "a" logo do not require this CO_2 treatment.
2. Dry ice placed in the desiccator may be used in lieu of a CO_2 vapor source. The powder may be somewhat damp and sticky after CO_2 exposure, but this does no harm.
3. When decanting in Step 3, the insoluble residue should be disturbed as little as possible as this may cause a suspension of $KHCO_3$ to form (which will then be extracted with clorazepate in the subsequent aqueous partition).
4. Evaporation of the aqueous solution (Step 6) may take 1 to 2 h, depending on the volume evaporated. Spectra of the monopotassium salt will be obtained, which may contain some water. Some of the absorptions will be broadened in comparison to those in the (undissolved) monopotassium clorazepate spectrum (see Discussion).
5. Abbott has recently marketed Tranxene tablets. While all of this work has been done with capsules, preliminary examination of these new tablets indicates that they probably contain the same excipients. This extraction procedure has been found to work well for powdered scrapings of these tablets as well; such tablets usually require a CO_2 treatment.
6. When this procedure is used for Tranxene SD time release tablets, two extraneous (unidentified) broad absorptions near 1600 and 1070 cm^{-1} result, in addition to those of clorazepate. The use of CM 9:1 results in spectra having much less of these, and should be used in place of CM 3:1 when analyzing Tranxene SD tablets.

Thin-Layer Chromatography (Table 3)

Solvent Systems—

1. Chloroform: methanol: concentrated NH_4OH, 30:10:3; saturated with KCl (excess KCl placed in developing jar along with solution and a filter paper lining, Note 1).
2. Ethanol: concentrated NH_4OH, 10:1; saturated with KCl (as above).
3. Methanol: concentrated NH_4OH, 10:1; saturated with KCl (as above).

Plates: Analtech Silica Gel GHL (10 by 2.5 cm, each plate).
Visualization: iodine saturated chamber.
Procedure—Place a small amount of capsule powder (or tablet scraping) in a small test tube and add several drops of the System 1 solvent. Mix *thoroughly*, centrifuge, and decant. Spot 0.5 to 1 mL of this solution. As reference, spot 0.5 mL of a (saturated) solution of monopotassium clorazepate prepared in the same manner.
Notes—

1. With most conventional solvent systems, clorazepate gives very pronounced tailing or bearding (tails that precede the sample). This is alleviated, to some extent, by the addition of

TABLE 3—*Thin-layer chromatography results for clorazepate and some other benzodiazepines.*

	Relative Migration Rates		
Solvent System	Clorazepate	*N*-desmethyldiazepam	Other Benzodiazepines Tested (Note 3)
1	0.13	0.80	>0.60
2	0.23	0.77	>0.60
3	0.69	0.83	>0.75

potassium chloride (or other salts) to the developing solution. The mechanism for this decrease in tailing has been attributed[19] to a binding, and saturation of, the most active sites of the stationary phase by the salt ions.

2. While *N*-desmethyldiazepam visualizes with I_2, stronger visualization for spots of this compound is obtained using acidified iodoplatinate spray. After removal of the plates from the I_2 chamber, such a visualization produces black spots for *N*-desmethyldiazepam and fainter yellow/brown spots for clorazepate.

3. Since the plates were not dried before use, or otherwise conditioned, the R_fs given are only approximately reproducible. The R_fs for some other benzodiazepines tested for Systems 1 and 2, respectively are: prazepam, 0.91, 0.81; flurazepam, 0.79, 0.90; diazepam, 0.78, 0.90; clonazepam, 0.77, 0.78; chlordiazepoxide, 0.73, 0.83; lorazepam, 0.62, 0.66; and oxazepam, 0.61, 0.67.

4. For System 1, the identity of the spots attributed to clorazepate were verified by infrared spectra of extracts of developed plate scrapings.

5. Except for System 1, extracts of Tranxene SD tablets using this procedure do not give good spots for clorazepate; the infrared procedure is better suited for these time release tablets.

6. Reverse phase plates (Analtech) using various water-methanol-ammonia mixtures were also tested and found to give clorazepate *N*-desmethyldiazepam spots migrating in an order opposite those above. These, too, gave spots with very pronounced bearding and tailing; addition of salt to these solutions, however, did not decrease this significantly and resulted instead in such plates reverting to behavior characteristic of regular (polar stationary phase) plates.

Summary

The interfering substances, which previously prevented an infrared analysis of clorazepate as present in the pharmaceutical products Tranxene and Azene, have been identified. They have been found to arise primarily from the various capsule ingredients which include talc, cellulose, $KHCO_3$, K_2CO_3, potassium hydroxide, potassium chloride, and stearate and palmitate salts. Older Tranxene capsules contain mostly talc and $KHCO_3$. They may also contain appreciable amounts of *N*-desmethyldiazepam and 2-amino-5-chlorobenzophenone, the clorazepate decarboxylation and hydrolysis products, respectively. These compounds form slowly in the presence of atmospheric CO_2 and moisture. Recently manufactured Azene and Tranxene, on the other hand, may contain appreciable amounts of potassium hydroxide and K_2CO_3, which are slowly neutralized by atmospheric CO_2 and moisture to $KHCO_3$.

The identification of these interferences was complicated by the transformations of the capsule ingredients with time, a manufacturer's change in the Tranxene capsule composition, and the chemical nature of these mostly inorganic ionic substances. No analytical distinction exists between dipotassium and monopotassium clorazepate, as solution of the former converts it to the latter. An extraction procedure is presented that effectively excludes all of the interfering substances, allowing infrared identification of clorazepate.

Acknowledgments

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[19]Analtech, Inc., private communication, 33rd Annual Meeting of the American Academy of Forensic Sciences, Los Angeles, CA, 17-20 Feb. 1981.

and especially Mrs. Ruth Seiwald, for their patience in typing the various drafts of this paper; and Mr. Ben Smith for his graphics assistance. We also wish to thank Mr. John Anderson and Mr. Joseph Gorski for lending us their ultraviolet spectrometer, and Abbott Laboratories for providing us with samples of monopotassium and dipotassium clorazepate and *N*-desmethyldiazepam.

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