Photo-Induced Isomerization of 8-(3,4,5-Trimethoxystyryl)caffeine as Possible Route of Drug Decomposition

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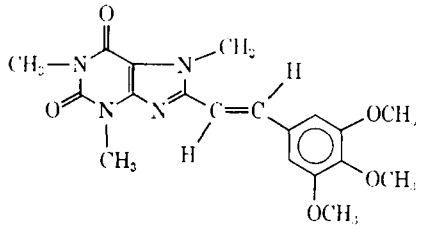
Abstract □ *trans*-8-(3,4,5-Trimethoxystyryl)caffeine, dissolved in methanol or chloroform, rapidly isomerizes to an equilibrium mixture of *trans*-*cis*-isomers in the presence of visible light. Geometric isomerization was established by catalytic hydrogenation of both reactant and product to yield 8-(3,4,5-trimethoxyphenethyl)-caffeine.

Keyphrases □ 8-(3,4,5-Trimethoxystyryl)caffeine—decomposition by photoisomerization □ Caffeine derivatives—decomposition of 8-(3,4,5-trimethoxystyryl)caffeine by photoisomerization □ Photoisomerization—decomposition of 8-(3,4,5-trimethoxystyryl)caffeine

trans-8-(3,4,5-Trimethoxystyryl)caffeine (I) is a caffeine derivative being evaluated for use as a cerebral stimulant. Past work (1–4) indicated that photoisomerization about an ethylenic double bond is a general reaction. Since this investigational drug is a styryl derivative containing an ethylenic moiety, it is possible that photo-induced isomerization could occur and cause subsequent developmental problems. The effect of visible light on the drug was investigated to obtain information in this area.



I

RESULTS AND DISCUSSION

Dilute solutions (c 0.00189%) of I were prepared and exposed to visible light for varying periods. Sequential UV spectra (Fig. 1) of such solutions changed very rapidly during initial exposure (0.5 hr.), after which they became essentially constant. Almost the same behavior was noted with either chloroform or methanol as the solvent. UV spectra of solutions stored at the same temperature in the dark did not change.

To determine the nature of the process responsible for the observed changes, reaction products were isolated for identification. Preparative TLC was without value in this regard since the products and reactant had the same R_f value in available chromatographic systems.

A quantity of crystalline reaction product (II) was obtained by exposing a solution of I in chloroform to the point of spectral redundancy. Its molecular weight, determined by mass spectroscopy and elemental analysis, was identical to that of the drug, thereby indicating that the drug and product are isomers.

Confirmation that this isomerization involved geometric conversion was obtained by saturating the olefinic double bond presumed to be present in the reaction product to obtain 8-(3,4,5-trimethoxyphenethyl)caffeine (III), a compound previously obtained[1] from I by the same hydrogenation procedure (Scheme I).

[1] D. Schweiss, Parke, Davis & Co., Ann Arbor, MI 48105, personal communication.

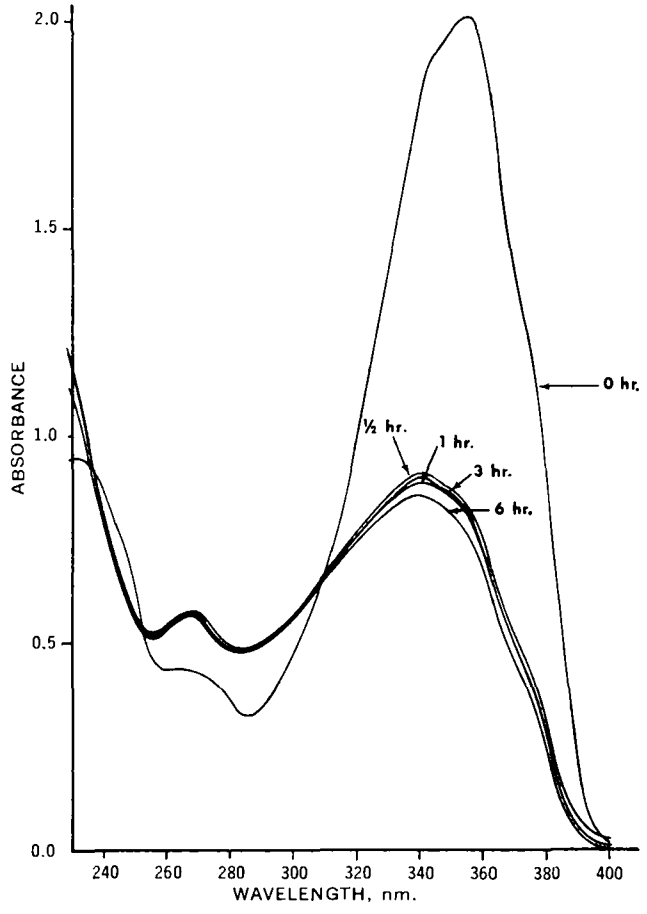


Figure 1– *Sequential UV spectra of I in methanol kept in the light cabinet. Exposure time (hours) is noted.*

Analysis of the UV spectra of a methanolic solution of I exposed to light to spectral redundancy indicated that an equilibrium mixture (80% *cis*–20% *trans*) is eventually formed. This finding was substantiated by similarly exposing the *cis*-isomer and noting that its spectrum changed to that corresponding to an 84:16 *cis*–*trans* mixture.

EXPERIMENTAL[2]

Initial Observations—One hundred milliliters of a 0.00189% solution of I in methanol was prepared and placed in a 100-ml. volumetric flask. The flask was placed in the light cabinet, and aliquots were removed periodically for UV determination.

Preparation and Identification of Reaction Product—One gram of I was dissolved in 3 l. of chloroform and kept in a light cabinet at 45°. The reaction was followed by obtaining repetitive UV spectra: when they ceased to change, the chloroform was removed and the residue was recrystallized twice from chloroform–methanol and finally from acetonitrile, m.p. 187–189° (I, m.p. 245–247°).

[2] The equipment was as follows: Cary model 14 S/W, steel light cabinet equipped with several 100-w. incandescent bulbs, Perkin-Elmer model 457 grating IR spectrophotometer, Varian A-60 NMR spectrometer; and Finnigan model 1015 quadrupole mass spectrometer.

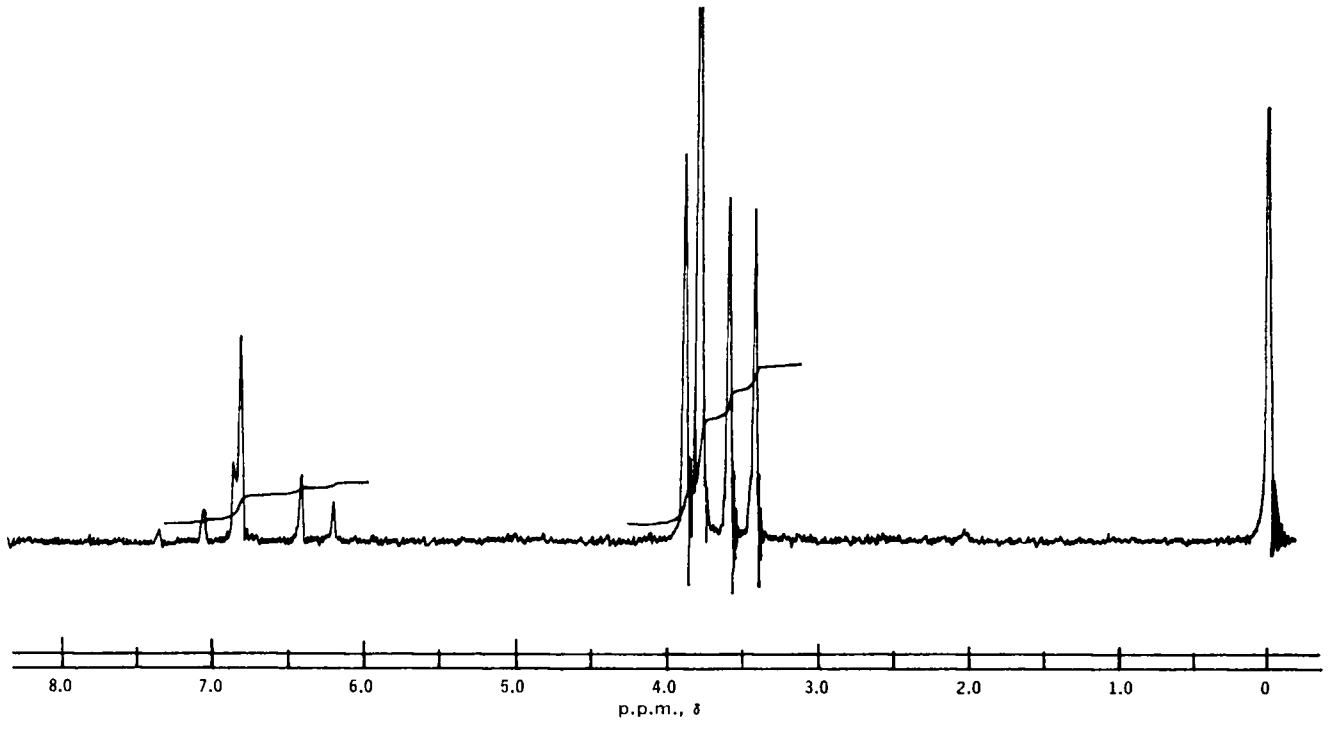


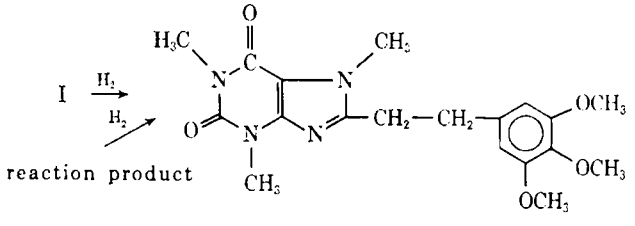
Figure 2—*NMR spectrum of II in $CDCl_3$.*

Anal.—Calc. for $C_{19}H_{22}N_4O_5$: C, 59.10; H, 5.70; N, 14.50. Found: C, 59.16; H, 5.65; N, 14.59.

The UV spectrum[3] showed $\lambda_{max}^{methanol}$ 327 and 268 nm. (ϵ 1.30 × 10^4 and 1.084 × 10^4, respectively).

The NMR spectrum (Fig. 2) showed a singlet at 3.75 (9H) p.p.m., assigned to the methoxy methyl protons; singlets at 3.41 (3H), 3.58 (3H), and 3.87 (3H) p.p.m., assigned to the three *N*-methyl group protons; and doublets centered at 6.95 (1H) and 6.31 (1H) p.p.m. (J = 13 Hz.), assigned to the vinyl protons[4].

Reduction of I and II Approximately 0.6 g. compound was suspended in 75 ml. of acetic acid in a Parr machine. Then 0.1 g. of 20% palladium-on-carbon was added. The hydrogen pressure was kept at 50 p.s.i. and the solution was shaken for 5 min. Then the solution was filtered and the solvent was evaporated. The residue was then suspended in 50 ml. of water, and the solution was made neutral by adding ammonium hydroxide. The precipitate was filtered and then recrystallized from ethanol. The reduction



III

Scheme I

products of I (A) and II (B) had identical properties and were identified as 8-(3,4,5-trimethoxyphenethyl)caffeine (III).

Properties of A and B are as follows:

	A	B
Melting point	141–143°	141–143°
UV (methanol) $\epsilon\lambda^{276}$	1.20×10^4	1.23×10^4
IR	identical spectra	

Anal.—Calc. for $C_{19}H_{24}N_4O_5$: C, 58.75; H, 6.23; N, 14.42. Found: (A) C, 58.35; H, 6.54; N, 14.18. (B) C, 58.24; H, 6.17; N, 14.32.

Determination of the Composition of Spent Reaction—The composition of the spent reaction was established by analysis of the UV spectrum obtained when the methanolic solution of the *trans*-isomer was exposed to spectral redundancy. The absorptivity (a) (1%, 1 cm.) at λ342 of the mixture was found to be 450, which corresponds to an 80:20 mixture (*cis-trans*), *i.e.*:

$$\%\ trans = \frac{a\,(1\%,\ 1\ cm.)_{obs} - 318}{659} \qquad \text{(Eq. 1)}$$

where 318 = $a\,(1\%,\ 1\ cm.)_{\lambda342}^{cis}$ and 659 = $\Delta a(1\%,\ 1\ cm.)_{\lambda342} = a(1\%,\ 1\ cm.)_{\lambda342}^{trans} - a(1\%,\ 1\ cm.)_{\lambda342}^{cis}$.

When a 0.002% solution of the *cis*-isomer was similarly exposed to a spectral redundancy, the a(1%, 1 cm.) at λ342 was found to be 425. This, in turn, indicates the composition of the equilibrium mixture to be 84:16, in reasonable agreement with that determined by starting with the *trans*-isomer.

CONCLUSION

Exposure of dilute solutions of *trans*-8-(3,4,5-trimethoxystyryl)-caffeine in methanol or chloroform to visible light results in rapid *trans-cis* isomerization; *trans-cis* isomerization proceeds with the ultimate formation of an equilibrium mixture containing approximately 82% *cis*-18% *trans*.

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[3] The UV parameters are in accord with previous observations (5–7) made on UV properties of *cis*- and *trans*-stilbenes in that the *trans*-isomer has the greater molar absorptivity and a maximum at longer wavelength than the *cis*-isomer (355 and 327 nm., respectively). Also, the molar absorptivity at 268 nm. is greater for the *cis*-isomer than for the *trans*-isomer, which is in accord with expectation.

[4] The coupling constant in I (15 Hz.) is less than normal for a vinyl hydrogen but is within the limit of 5–30 Hz. In I (*trans*), there is conjugation from the benzene ring through the vinyl system and then through the purine moiety. This reduces bond order between the carbons and thus the value of J. In II (*cis*), steric hindrance prevents both the benzene ring and purine ring from being simultaneously coplanar with the ethylene system and thus should markedly reduce conjugation. This, in turn, raises bond order in the ethylene and thereby increases J. The overall result is to bring the values of J for the *cis*- and *trans*-isomers closer together than normal.

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(5) A. Gillam and E. Stern, "Electronic Absorption Spectroscopy," Richard Clay and Co., Bungay, Suffolk, England, 1957, p. 267.
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Application of Conformation-Dependent Antibodies: Enhancement of Enzyme Activity of L-Glutamate Dehydrogenase [L-Glutamate NAD(P) Oxidoreductase (Deaminating) EC 1.4.1.3]

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Abstract □ By using a nonprecipitating system, it was found that the enzyme activity of L-glutamate dehydrogenase [L-glutamate NAD(P) oxidoreductase (deaminating) EC 1.4.1.3] (I) is enhanced by the addition of small amounts of anti-I-serum. It is suggested that this enhancement is due to the ability of conformation-dependent antibodies to maintain L-glutamate dehydrogenase in a more favorable conformation and thereby influence the rate of conversion of sodium α-ketoglutarate to sodium glutamate.

Keyphrases □ L-Glutamate dehydrogenase [L-glutamate NAD(P) oxidoreductase (deaminating) EC 1.4.1.3]—immunochemical properties □ Antibodies, L-glutamate dehydrogenase [L-glutamate NAD(P) oxidoreductase (deaminating) EC 1.4.1.3] produced—specificity □ Enzyme activity—enhancement of L-glutamate dehydrogenase

It has been shown that the enzymatic activity of L-glutamate dehydrogenase [L-glutamate NAD(P) oxidoreductase (deaminating) EC 1.4.1.3] (I) is dependent upon the conformation of the molecule (1–7). Immunochemical studies with this enzyme, using a precipitating system, showed that rabbit antibodies possess specificities not only for the primary structure but also for the conformation of the antigen (8, 9).

The effect of conformation-dependent antibodies on their homologous antigens is of interest. With respect to I, it was reported (10) that normal rabbit serum activates purified preparations of human and bovine I by about 40% in reactions using α-ketoglutarate and glutamate as substrates. It was also reported (10) that anti-I-serum enhanced the enzyme activity of I using α-ketoglutarate as substrate. It was, therefore, of interest to extend the reported investigations by studying the effect produced by these conformation-dependent antibodies over a period of time on the enzyme activity.

EXPERIMENTAL

Nicotinamide adenine dinucleotide reduced (NADH), α-ketoglutarate, and bovine liver L-glutamate dehydrogenase [L-glutamate NAD(P) oxidoreductase (deaminating) EC 1.4.1.3] were purchased[1]; the latter material was obtained as a crystalline suspension in ammonium sulfate. Compound I was prepared for use by dialyzing against a standard buffer solution (0.03 *M* Na_2HPO_4–0.01 *M* NaH_2PO_4–1×10^{-3} *M* disodium edetate), pH 7.4.

Immunochemistry—Ten rabbits were treated at weekly intervals with 5 mg. of I. The first 3 weeks they were injected intradermally, using complete Freunds adjuvant as the suspension medium. The rabbits were bled the following week using the standard heart puncture technique. Thereafter, the rabbits were bled and immunized on alternate weeks. Incremental amounts of I were added to 1-ml. aliquots of the antiserum obtained from each rabbit. These mixtures were incubated at 37° for 1 hr. and then stored at 4° for 48 hr. Each rabbit antiserum gave a precipitin reaction, and the amount of protein precipitated was quantitated by analysis for nitrogen (Kjeldahl).

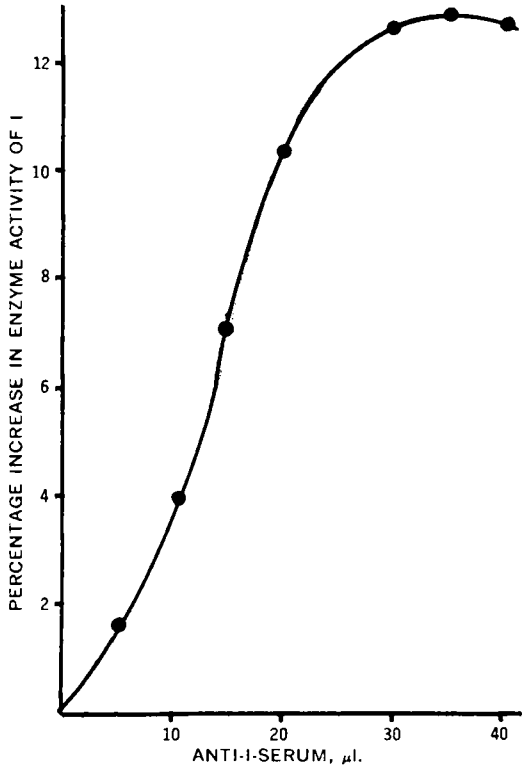

Figure 1—*Percentage increase in enzyme activity of I in the presence of anti-I-serum.*

[1] Sigma Chemical Co.