with the blue tetrazolium procedure (Table I, footnote *i*), presumably due to the heat used to extract the active ingredient. An extraction procedure using cold alcohol gave inconsistent and sometimes low results.

Another advantage of the developed method is that some inactive ingredients can be estimated without additional cost. For example, in commercial injections, parabens were estimated to be only between 76 and 80% of the label claim (Table I, footnotes *a* and *b*). This finding is presumably due to paraben adsorption onto the rubber closures. This problem was not recorded with benzyl alcohol (Table I, footnote *c*) as the preservative.

## REFERENCES


(1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. 122–126.

(2) J. A. Mollica and R. F. Strusz, *J. Pharm. Sci.*, **61**, 444 (1972).

(3) A. M. DePaolis, G. Schnabel, S. E. Katz, and J. D. Rosen, *J. Assoc. Off. Anal. Chem.*, **60**, 210 (1977).

(4) J. C. K. Loo and N. Jordan, *J. Chromatogr.*, **143**, 314 (1977).

(5) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 138.


## ACKNOWLEDGMENTS


The author thanks Merck Sharp & Dohme for the generous supply of dexamethasone and dexamethasone sodium phosphate powders.


# Terpenoid Biotransformation in Mammals II: Biotransformation of *dl*-Camphene in Rabbits


**T. ISHIDA *[x], Y. ASAKAWA [‡], T. TAKEMOTO [‡], and T. ARATANI [§]**



Received September 28, 1978, from the **Hiroshima Institute of Technology, Itsukaichi, Hiroshima 738, Japan*, the [‡]*Institute of Pharmacognosy, Tokushima-Bunri University, Tokushima 770, Japan*, and the [§]*Faculty of Integrated Arts and Sciences, Hiroshima University, Hiroshima 730, Japan.* Accepted for publication January 11, 1979.



**Abstract** □ The biotransformation of *dl*-camphene in rabbits was investigated. Four neutral metabolites, 6-*exo*-hydroxycamphene, 10-hydroxycamphene, and diastereoisomers of camphene-2,10-glycol, were identified and two alcohols, 7-hydroxycamphene and 3-hydroxytricyclene, were estimated by IR, UV, NMR, and mass spectra and chemical degradations. The formation of these compounds can be explained through a homoallylic oxidation or an epoxide formation.



**Keyphrases** □ Camphene—biotransformation in rabbits, four urinary metabolites identified by spectrometry and chemical degradations □ Biotransformation—camphene in rabbits, four urinary metabolites identified by spectrometry and chemical degradations □ Choleretic activity—camphene, biotransformation in rabbits, four urinary metabolites identified by spectrometry and chemical degradations


During investigations into terpenoid detoxification in mammals, the biotransformation of 3-carene and $\alpha$- and $\beta$-pinenes having a *gem*-dimethyl group on the three- or four-membered ring, respectively, was reported (1). In this paper, the biotransformation of camphene in rabbits is reported. Camphene has a *gem*-dimethyl group on the five-membered ring.

Camphene is found in the essential oils of most plants, and conifers containing this compound are often damaged worldwide by field animals. The choleretic activity of camphene in rats has been studied (2). The present investigation was carried out to clarify camphene biotransformation in mammals with respect to xenobiotics.

## RESULTS

**Characterization and Identification of Free Neutral Metabolites**—TLC and GLC revealed the products in the urinary extracts as shown in Fig. 1. The metabolites were column chromatographed on silica gel in *n*-hexane with gradually increasing amounts of ethyl acetate. When necessary, metabolites were isolated by preparative GLC.

**Metabolites 1-3 (M-I–M-III)**—Peak 2 was isolated preparatively by GLC as one component (TLC and GLC) and had a fragrant odor; mass spectrum: *m/e* (%) 152 ($M^+$, $C_{10}H_{16}O$, 3), 134 (9), 119 (24), 108 (base), 93 (67), 72 (21), and 66 (28); IR: $\nu$ ($CHCl_3$) 3625, 3450, and 890 $cm^{-1}$. Its NMR spectrum showed two kinds of metabolites (M-I and M-II) in a 2:1 ratio. The major signal group assigned was: $\delta$ ($CDCl_3$) 4.84 and 4.63 (each 1H, s, *endo*-methylene), 3.80 [1H, q, $J_{5\text{-}exo\text{-}6\text{-}endo} = 7$, $J_{5\text{-}endo\text{-}6\text{-}endo} = 3$ Hz, —C*H*(OH)], 2.62 (1H, b, bridge head), 2.21 (1H, octet, $J_{5\text{-}exo\text{-}5\text{-}endo} = 13$, $J_{5\text{-}exo\text{-}6\text{-}endo} = 7$, $J_{5\text{-}exo\text{-}4} = 2$ Hz, *exo*-5), 1.89 (1H, b, *endo*-5), 1.63 (2H, b), and 0.98 and 1.04 (each 3H, *gem*-dimethyl). All of these M-I signals agreed well with those of the synthetic 6-*exo*-hydroxycamphene (I) (3).

The Jones oxidation of I yielded 6-oxocamphene [$\nu$ ($CHCl_3$) 1740 and 890 $cm^{-1}$] (4) with a minor ketone; mass spectrum (minor ketone): *m/e* (%) 150 ($M^+$, $C_{10}H_{14}O$, 25), 135 (12), 121 (15), 108 (41), 107 (98), 93 (base), 91 (54), and 79 (41); IR: $\nu$ ($CHCl_3$) 1740 and 890 $cm^{-1}$; NMR: $\delta$ ($CDCl_3$) 4.98 and 4.76 (each 1H, s), 3.08 (1H, b), and 1.16 and 1.07 (each 3H, s). These spectra revealed the minor ketone to be 7-oxocamphene. Therefore, 7-hydroxycamphene (II) (M-II) was estimated as the camphene metabolite.

The minor signal group found in the I NMR spectrum can be attributed to II: $\delta$ ($CDCl_3$) 4.73 and 4.48 (each 1H, s), 4.24 (1H, bs), 2.68 (1H, bs), and 1.04 (6H, s). The other minor ketone was obtained as the Jones oxidation product from M-III following M-I. Its spectra were considerably different from those of the above-mentioned ketones; mass spectrum: *m/e* (%) 150 ($M^+$, $C_{10}H_{14}O$, 7), 122 (16), 107 (base), 105 (12), 91 (28), and 79 (14); IR: $\nu$ ($CHCl_3$) 1740 $cm^{-1}$; NMR: $\delta$ ($CDCl_3$) 1.21, 1.04, and 1.00 (each 3H, s). Disappearance of the *endo*-methylene group suggests some camphene rearrangement. Accordingly, this ketone was assigned as 3-ketotricyclene

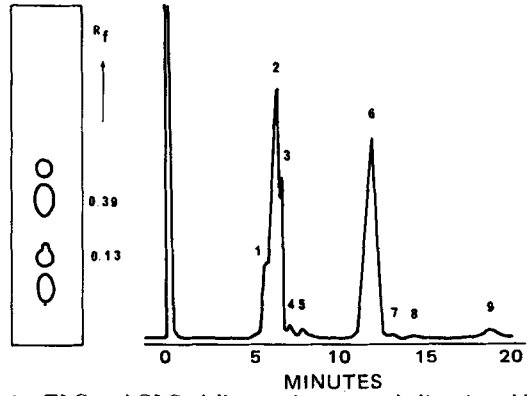
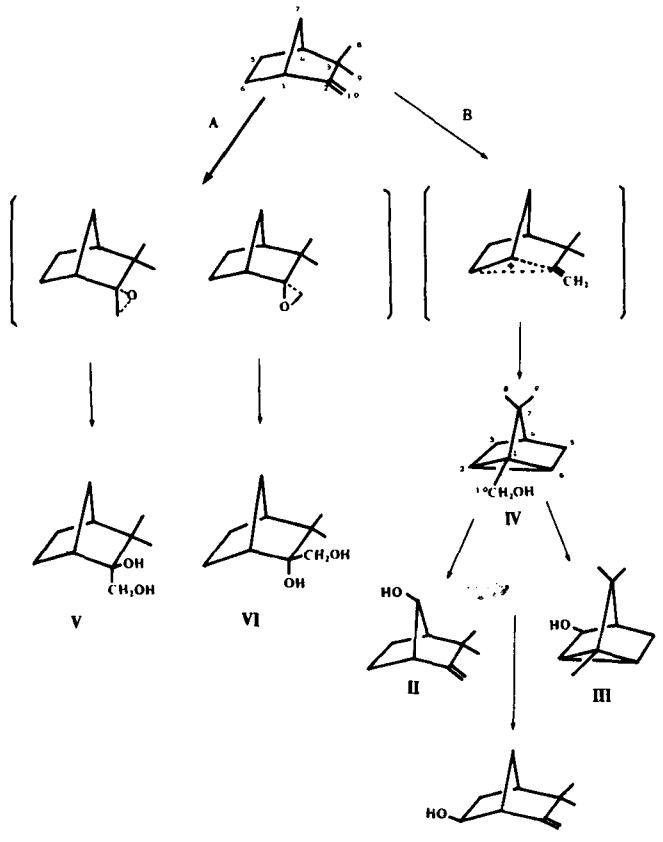



**Figure 1**—*TLC and GLC of* dl-*camphene metabolites in rabbits. The TLC solvent system was benzene–n-hexane–ethyl acetate (14:5:6); GLC utilized an SE-30 (5%) column.*




*0022-3549/79/0700-0928$01.00/0*



*Scheme I—Proposed* dl-*camphene metabolic pathway in rabbits. Pathway A is camphene epoxidation followed by cleavage of the oxirane ring by hydration. Pathway B consists of the formation of the nonclassical cation and cation rearrangement followed by hydroxylation.*

from those spectra. The corresponding alcohol, 3-hydroxytricyclene (III) (M-III), might be produced from camphene in rabbits. The peak 2 fragrance was due to I.

**Metabolite 4 (M-IV)**—Peak 1 also was isolated by preparative GLC. The mass spectrum of M-IV was: *m/e* (%) 152 ($M^+$, $C_{10}H_{16}O$, 35), 121 (49), 108 (base), 85 (72), and 79 (65); IR: $\nu$ ($CHCl_3$) 3640, 3455, 3070, and 850 $cm^{-1}$; NMR: $\delta$ ($CDCl_3$) 3.75 (2H, ss), $A_2B_2$ at 1.79, 1.68, 1.17, and 1.05 (each 1H, s); NMR: $\delta$ (dimethyl sulfoxide-$d_6$) 4.71 (t, $J$ = 5 Hz, 1H, a primary hydroxyl). From these results, M-IV was identified as 10-hydroxytricyclene (IV).

**Metabolite 5 (M-V)**—The $R_f$ 0.1 spot was in accord with peak 6, which was isolated by column chromatography. The mass spectrum of M-V was: *m/e* (%) 170 ($M^+$, $C_{10}H_{18}O_2$, 27), 152 (6), 139 (base), 121 (13), 101 (50), and 87 (60); IR: $\nu$ ($CHCl_3$) 3450 $cm^{-1}$. The NMR spectrum of the M-V acetate showed signals at $\delta$ 4.20 (in chloroform-$d_1$, methylene group adjacent to acetoxyl) and 3.27 (in dimethyl sulfoxide-$d_6$, s, *tert*-hydroxyl group) and clarified that M-V is a primary-tertiary diol.

This diol was oxidized by sodium periodate with sulfuric acid in ethanol and gave a ketone, camphenylon (VIII); IR: $\nu$ ($CHCl_3$) 1740 $cm^{-1}$. The NMR spectrum of VII showed only two signals of *gem*-dimethyl at $\delta$ 1.07 and 1.05. On the other hand, the spectrum of M-V showed four tertiary methyls [$\delta$ 1.02 and 0.98 (each 3H) and 0.96 (6H)] and two kinds of hydroxymethylene groups at $\delta$ 3.64 and 3.59. From these observations, it was decided that M-V consisted of two C-2 epimers (1:1) as shown in V and VI. The presence of diastereoisomers also was demonstrated by the remarkable melting-point difference; camphene-2,10-glycol obtained from rabbit urine had a melting point of 111–112° whereas that of 2-*exo*-hydroxycamphene glycol was reported as 196–198° (5).

## DISCUSSION

The following camphene metabolic pathway (Scheme I) in rabbits can be proposed from the metabolites.

On the basis of the good glycol yields (260 mg), pathway A was assumed to be the main route. Glycol formation through epoxides has been demonstrated in many other metabolic pathways (6). The homoallylic camphene oxidation products (I–IV) apparently were formed through the nonclassical cation as the intermediate, as shown in pathway B. The ketone (VII) showed the negative optical rotation, $[\alpha]_D$ −8°, and the negative circular dichroism curve ($\Delta\epsilon$ −0.12) (Fig. 2*a*). On the other hand, the synthetic (+)-camphenylon was reported to give the positive circular dichroism curve ($\Delta\epsilon$ +0.50) (7).

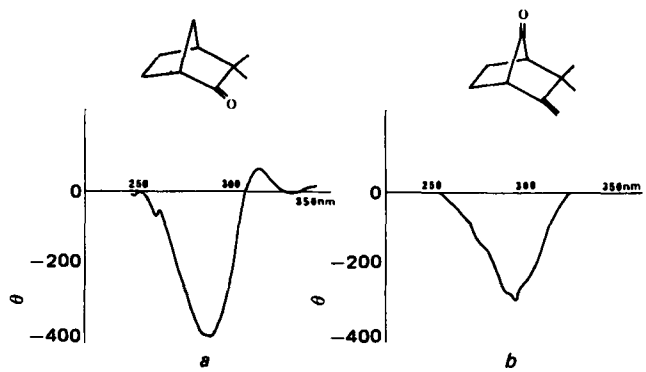


**Figure 2**—*Circular dichroism curves of camphenylon* (a) *and 7-oxocamphene* (b). *The concentrations were 3.77 × $10^{-2}$ M in ethanol* (a) *and 6.07 × $10^{-2}$ M in chloroform* (b).

Based on these results, the ratio was calculated as (−)/(+) = 0.6:0.4. Formation of the (−)-enantiomer from (−)-camphene was assumed to be more likely than formation of the (+)-enantiomer from (+)-camphene. Therefore, it was surmised that the degradation of (+)-camphene is faster than that of (−)-camphene and/or that the conversion of (+)-camphene into (−)-camphene may partly occur in rabbits just as in cicroprofen metabolism in rats (8). The negative circular dichroism curve of 7-oxocamphene (Fig. 2*b*) supports the proposal. The hydroxyl group orientation in II is considered to be *anti*- to the *gem*-dimethyl due to the steric factor.

## EXPERIMENTAL[1]

**Animals and Dosing**—Five male albino rabbits[2], 2–3 kg, were starved for 2 days before the experiments. Camphene (10 g) was dissolved in water (90 ml) containing polysorbate[3] (0.1 g) and homogenized for 20 min. This solution (20 ml), followed by water (20 ml), was administered to each rabbit through a stomach tube. This camphene dose corresponds to 800 mg/kg, and the total amount of camphene fed to all rabbits equaled 8 g.

The animals were housed in individual stainless steel metabolism cages and were allowed rabbit food[4] and water *ad libitum*. The urine was collected daily for 3 days after drug administration and stored at 0–5° until use.

**Extraction and Fractionation of Free Neutral Metabolites**—The urine was centrifuged at under 3000 rpm at 0° to remove feces and hairs, and the supernate was used for experiments. The urine was adjusted to pH 4.7 with acetate buffer and then incubated with $\beta$-glucuronidase–arylsulfatase (3 ml/1000 ml of the fresh urine) at 37° for 48 hr followed by continuous ether extraction for 48 hr. The ether extracts were washed with 5% $NaHCO_3$ and 5% NaOH to remove the acidic and phenolic fractions, respectively, and dried over magnesium sulfate. Ether was evaporated under reduced pressure to give free neutral metabolites (1.471 g).

---

[1] *dl*-Camphene (above 90%) (Tokyo Kasei Kogyo), $\beta$-glucuronidase–arylsulfatase (Boehringer Manheim), Wako gel 200, and reagent grade solvents (Wako Chemicals) were used. A Shimadzu GC-6A gas chromatograph was equipped with a hydrogen flame-ionization detector and a thermal conductive detector. For neutral metabolite detection, the following were used: a 2-m × 2.6-mm i.d. glass column, 5% SE-30 or 5% OV-1 on Diasolid L (60–80 mesh); an injector at 250°; an oven programmed at 100–250° at 10°/min; a detector at 250°; and a nitrogen flow rate of 40 ml/min. For preparative use, the same size glass column of 5% DEGS on Diasolid L (60–80 mesh, AW-DMCS) (Japan Chromato) was employed under the same conditions.

TLC plates (20 × 20 cm) were glass, precoated with silica gel 60 F-254, and were 0.25 cm thick (Merck). The solvent system was benzene–ethyl acetate–*n*-hexane (14:5:6 v/v). The chromatograms were visualized with iodine vapor and under UV light (254 nm). NMR spectra were recorded on a Hitachi R-22 or a Varian T-60 spectrometer. IR spectra were run in chloroform using a Hitachi 215 spectrometer. Mass spectra were obtained on a Shimadzu LKB-9000B. UV spectra were obtained on a Shimadzu US 200S. Circular dichroism spectra were recorded on a JASCO ORD/UV-5 spectrometer with a circular dichroism attachment.

[2] Japanese White, Miyamoto Jikken Dobutsu.

[3] Tween 80.

[4] Oriental RC-4.

**6-Oxocamphene from M-I**—M-I (9 mg) was dissolved in acetone (1 ml). Anhydrous chromic acid (1.33 g) was added to the dilute sulfuric acid (1.15 g in 2 ml of water) and well stirred. This oxidant (0.1 ml) was added by drops to the acetone solution at 0°, and the reaction mixture was shaken for 20 min. After 13 hr, $R_f$ 0.41 of M-I changed to 0.58. The reaction mixture was neutralized with 5% NaOH, extracted with methylene chloride (1 ml), and dried over magnesium sulfate. Evaporation of this solvent gave 6-oxocamphene (2.4 mg); mass spectrum: *m/e* (%) 150 ($M^+$, 54), 135 (24), 107 (base), 106 (78), 93 (40), 91 (60), 79 (34), and 77 (21); NMR: δ ($CDCl_3$) 5.07 and 4.82 (each 1H, s), 3.09 (1H), and 1.21 and 1.11 (each 3H, s).

**Camphene-2,10-glycol 10-Acetate from M-V**—Acetylation of M-V (100 mg) with acetic anhydride and pyridine gave its monoacetate (84.7 mg); mass spectrum: *m/e* (%) 214 ($M^+$, 4), 194 (13), 171 (82), 152 (78), 137 (53), 109 (base), and 43 (77); IR: ν ($CHCl_3$) 3550 and 1730 $cm^{-1}$; NMR: δ ($CDCl_3$) 4.20 (2H, s), 2.08 (3H, s), and 1.03 and 0.93 (each 3H, s); NMR: δ (dimethyl sulfoxide-$d_6$) 4.03 (2H, s), 2.03 (3H, s), 0.93 and 0.88 (each 3H, s), and 3.27 (1H, s, exchanged with deuterium oxide). The anhydrous ethereal solution (10 ml) of lithium aluminum hydride (38.8 mg) was added to the ethereal solution (20 ml) of the monoacetate (80 mg) under a nitrogen atmosphere and stirred at room temperature for 4 hr. Decomposition of the excess lithium aluminum hydride with ethyl acetate, filtration through a short column packed with magnesium sulfate, and evaporation of the solvent recovered camphene-2,10-glycols.

**Camphenylon (VII) from M-V**—The ethanol solution (5 ml) of M-V (100 mg) was added to the sodium periodate (130 mg)–1 *N* sulfuric acid solution (6 ml) at 40° and stirred for 13 hr until it became transparent (9). The reaction mixture was neutralized with 5% $NaHCO_3$, extracted with ether, and dried over magnesium sulfate. Evaporation of the solvent gave camphenylon (VII) (23.0 mg); mass spectrum: *m/e* (%) 138 ($M^+$, 28), 95 (10), 81 (5), 72 (14), 69 (base), 67 (67), and 41 (31); UV: $\lambda_{max}$ (ethanol) 287 (ε 24) and 240 (21) nm; $[\alpha]_D$ −8.3° (c 2.4, $CHCl_3$).

## REFERENCES


(1) T. Ishida, Y. Asakawa, M. Okano, and T. Aratani, *Tetrahedron Lett.*, **1977**, 2437.

(2) K. Moersdorf, *Chim. Ther.*, **1966**, 442.

(3) M. Julia, D. Mansuy, and P. Detraz, *Tetrahedron Lett.*, **1976**, 2141.

(4) Y. Uchio, thesis.

(5) P. Hirsjarvi, D. Klenberg, M. Patala, and P. Eenila, *Suom. Kemistil.*, **34B**, 152 (1961).

(6) J. W. Regan and L. F. Bjeldane, *J. Agr. Food Chem.*, **24**, 377 (1976). R. P. Hopkins, C. J. W. Brooks, and L. Young, *Biochem. J.*, **82**, 457 (1962). R. Miura, S. Honmura, and M. Nakazaki, *Tetrahedron Lett.*, **1968**, 5271.

(7) C. Coulombeau and A. Rassat, *Bull. Soc. Chim. Fr.*, **1966**, 3752.

(8) A. V. Dean, S. J. Lan, K. J. Kripalani, L. T. Difazio, and E. C. Schreiber, *Xenobiotica*, **7**, 549 (1977).

(9) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Wiley, New York, N.Y., 1967, p. 815.


## ACKNOWLEDGMENTS


The authors thank Dr. Y. Hirose, Institute of Food Chemistry, for the mass spectra and Dr. M. Julia, Centre National de la Recherche Scientifique, for the NMR spectrum of 6-*exo*-hydroxycamphene.


# New Compounds: Arylsulfonylhydrazones Derived from Various Heterocycles


**RICHARD JUNEAU *x, SCOTT MITCHELL *, and GARY WOLGAMOTT ‡**



Received November 20, 1978, from the **Department of Medicinal Chemistry, School of Pharmacy, and the* ‡*Department of Microbiology, Southwestern Oklahoma State University, Weatherford, OK 73096.* Accepted for publication January 24, 1979.



**Abstract** □ Ten arylsulfonylhydrazones were prepared from various heterocycles. The antimicrobial activity of these compounds was investigated, as was the cytotoxic activity of one of them.



**Keyphrases** □ Arylsulfonylhydrazones—derived from various heterocycles, antimicrobial and cytotoxic activity □ Hydrazine—derivatives, various heterocycles, antimicrobial and cytotoxic activity □ Antineoplastic agents, potential—arylsulfonylhydrazones, derived from various heterocycles, antimicrobial and cytotoxic activity

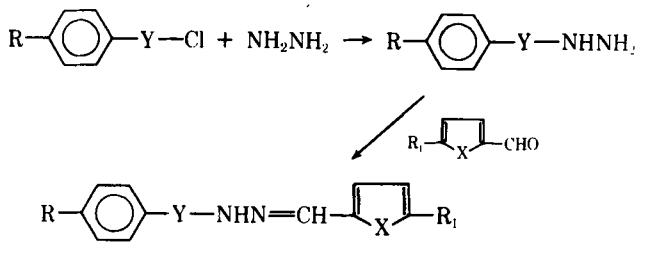

Arylsulfonylhydrazones of 2-formylpyridine and its oxide have been investigated as cytotoxic agents (1). Since studies involving the preparation and biological properties of arylsulfonylhydrazones derived from aromatic heterocycles other than pyridine and quinoline are not numerous, the synthesis and antibacterial screening of the compounds listed in Table I were attempted.

## DISCUSSION

**Chemistry**—The compounds listed in Table I were synthesized by standard procedures, consisting of reaction between hydrazine and *p*-toluenesulfonyl chloride or α-toluenesulfonyl chloride followed by condensation with the appropriate heterocyclic 2-carboxaldehyde (Scheme I). For IV and IX, the requisite 5-nitrofurfural was obtained by the acid hydrolysis of 5-nitrofurfurylidene diacetate according to a literature method (2). Compounds IV, VIII, and IX are not new compounds and were reported previously (3–5).

The hydrazone linkage in the synthesized compounds allows geometric isomers to be formed. Attempts to detect *E*- and *Z*-isomers by TLC in several solvent systems were successful only with III and VII. Compounds III and VII demonstrated the presence of a trace amount of a second compound. However, only in the case of III were the two compounds nonoverlapping and amenable to dry column chromatography.

The IR and NMR spectra of III*a* and III*b* were practically superimposable and offered no aid in configurational assignments. Compound III*a* showed a bathochromic shift in the UV spectrum of only 3 nm (285–288) in going from a polar to a nonpolar solvent (ethanol to benzene), whereas III*b* showed a bathochromic shift of 10 nm (272–282). These data suggest the *Z*-configuration for III*b*, the trace component and more mobile compound on TLC (silica gel, chloroform), and the *E*-configu-




R = H, $CH_3$; Y = $SO_2$, $CH_2SO_2$;
X = O, N, S; $R_1$ = H, $NO_2$

*Scheme I*




*0022-3549/79/0700-0930$01.00/0*