lowed by increased ability to antagonize tremors and lacrimation induced by tremorine.

Inhibition of the Chromodacryorrhea Induced by Acetyl-β-methylcholine (Mecholyl).—Compound VII antagonized the action of Mecholyl on the parasympathetic effector cells of the accessory lacrimal glands in the rat. The median protective dose (PD_{50}) of compound VII was 1.4 mg./Kg., while that for atropine was shown to be 0.3 mg./Kg. In this test, compound VII was approximately one-fifth as active as atropine, but only slightly less active than CDS-216 (PD_{50} of 0.9 mg./Kg.).

Mydriatic Action.—Compound VII, when applied topically to the cornea of the rabbit, did not have effect on pupillary diameter at concentrations to 1.0%. CDS-216, at a concentration of 1.0%, caused only slight mydriasis; atropine, at a concentration of 0.5%, produced maximal pupillary dilation.

Effect on Hexobarbital Hypnosis.—When administered at several dose levels, compound VII did not alter either the time of induction or the duration of sleep produced by hexobarbital.

Other Pharmacologic Actions.—Compound VII was devoid of significant analgesic activity and possessed no local anesthetic action. It exerted neither an adrenolytic effect nor a potentiating action on the pressor response to *l*-epinephrine, and did not antagonize the vasodepressor action of histamine. At the dose levels employed, compound VII appeared to potentiate the action of serotonin on the isolated rat uterus.

EXPERIMENTAL[1]

α-Phenyl-α-3-thenylacetic Acid.—This agent was prepared by standard malonic ester synthesis, namely by refluxing 24.1 Gm. (0.1 mole) of the sodium salt of phenyldiethylmalonate with 17.6 (0.1 mole) of 3-thenylbromide (8) in ethanol. After refluxing for 12 hours, the reaction mixture was filtered and the solvent evaporated. The residue was then saponified with alcoholic KOH and the alcohol evaporated and the residue acidified with hydrochloric acid. The resulting mixture was then extracted twice with 100 ml. of benzene and dried over potassium carbonate. After filtering and evaporating the benzene, the residue was heated in high-vacuum to yield a semisolid. Recrystallization of this material from benzene-petroleum ether gave crystals melting at 93°; yield, 38%.

Anal.—Calcd. for $C_{13}H_{12}O_2S$: C, 67.25; H, 5.24; S, 13.80. Found: C, 67.32; H, 5.27; S, 13.72.

β-(α-Phenyl-α-3-thenylacetoxy) Ethyldimethylsulfonium Bromide.—Ten grams (0.04 mole) of α-phenyl-α-3-thenylacetic acid was reacted with 0.92 Gm. (0.04 mole) of sodium in 75 ml. of isopropanol. This was then refluxed for 30 minutes and 7.0 Gm. (0.06 mole) of β-chloroethylmethylsulfide added over a period of 30 minutes with good stirring. The reaction mixture was then refluxed for 24 hours; the salt formed was separated by filtration and the solvent removed by distillation. The residue was dissolved in 20 ml. of methanol and 15 Gm. (0.16 mole) of liquid methylbromide added. The reaction mixture was then allowed to stand at room temperature for 5 days in a sealed container. After boiling away both the methanol and the unreacted methylbromide, the residue was triturated with anhydrous ether. A crystalline product was formed in 72% yield; m.p. 100–101°.

Anal.—Calcd. for $C_{17}H_{21}BrO_2S_2$: C, 50.87; H, 5.27; Br, 19.19; S, 15.98. Found: C, 50.72; H, 5.36; Br, 20.10; S, 15.71.

SUMMARY AND CONCLUSIONS

Substitution of the cyclohexenyl group of compound CDS-216 with a methylthiophene grouping affected adversely the antispasmodic activity and did not alter the toxicity appreciably.

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[1] Microanalysis performed by G. Roberts, Florham Park, N. J. All melting points are uncorrected.

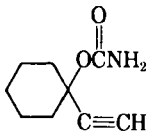
Identification of Ethinamate, Ethchlorvynol, and Methylparafynol

By W. N. FRENCH

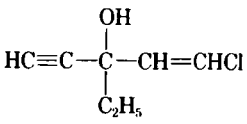
Procedures for the identification of ethinamate, ethylchlorvynol, and methylparafynol by derivative formation are described. The acid catalyzed rearrangement of ethchlorvynol gave 3-ethyl-2-penten-4-ynal.

FEW PROCEDURES are available in the literature for the identification of ethinamate (I), ethchlorvynol (II), and methylparafynol (III).

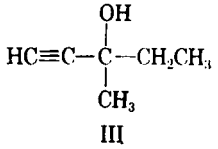
Ethinamate has been identified by general tests for unsaturation and the presence of a terminal



I



II



III

Received September 6, 1963, from the Pharmaceutical Chemistry Section, Food and Drug Directorate, Ottawa, Ontario, Canada.
Accepted for publication October 16, 1963.

TABLE I.—2,4-DINITROPHENYLHYDRAZONE DERIVATIVES

Parent Compd.	Crude Product Yield, %	Crude Product M.p., °C.	Crystallized Product M.p., °C.	Crystallized Product $\lambda_{max.}$ (EtOH)	Crystallized Product ϵ[d]
Ethinamate	50	196–199	204–205[a]	373	26,800[b]
Ethchlorvynol	70	119–121	123–124	375	32,800
Methylparafynol	35	180–185	195–196[c]	370	26,500[b]

[a] Reported, m.p. 204° (9) and 199–201° (10). [b] Reported value, $\lambda_{max.}$ at 377 mμ, ϵ = 26,000 (9). [c] Reported, m.p. 198.5° (9). [d] In this table and subsequent discussion, ϵ is defined as the molecular extinction coefficient, *i.e.*, the absorbance of a 1 *M* solution.

acetylenic group (1), by chromatographic (2–4) and crystallographic (5) techniques, and by derivative formation (6). The infrared spectrum of ethchlorvynol has been published (7), and methylparafynol has been characterized by derivative formation (8, 9).

This paper describes some procedures permitting the identification of the above compounds both in pure form and in commercial preparations.

EXPERIMENTAL

2,4-Dinitrophenylhydrazine Reagent.—Slowly add 25 ml. of water to a solution of 2.0 Gm. of 2,4-dinitrophenylhydrazine in 20 ml. of sulfuric acid while gently swirling and cooling the flask contents in a water bath. Dilute with 50 ml. of alcohol.

Preparation of 2,4-Dinitrophenylhydrazone Derivatives.—Dissolve 100 mg. of the acetylenic compound in 10 ml. of 2,4-dinitrophenylhydrazine reagent and place in a water bath at 50–55° for 4 minutes (ethchlorvynol), 1 hour (ethinamate), or 3 hours (methylparafynol). Remove the solution and let stand at room temperature for 30 minutes. Filter off the reaction product with suction and wash with 70% ethanol. Crystallize the derivative from ethyl acetate (ethinamate) or from ethanol (methylparafynol and ethchlorvynol). The results are shown in Table I.

Preparation of *p*-Nitrobenzoate Esters of Ethchlorvynol and Methylparafynol.—Add 1 *M* equivalent of *p*-nitrobenzoic acid (for ethchlorvynol, 230 mg.; for methylparafynol, 340 mg.) to a solution of 200 mg. of the acetylenic carbinol in 5 ml. of dry pyridine and warm the mixture on a steam bath to dissolve the carboxylic acid. Add 2 *M* equivalents of *p*-toluenesulfonyl chloride (for ethchlorvynol, 525 mg.; for methylparafynol, 780 mg.) and heat the solution on a steam bath for 40 minutes. Cool the solution, and pour with stirring into 50 ml. of ice-water mixture. Filter off the brown solid, wash with water, and crystallize from aqueous alcohol. Ethchlorvynol yields 0.35 Gm. (87%) of reaction product, m.p. 87.5 to 88.0°. On recrystallization, the melting point is raised to 89.0 to 89.5°. Methylparafynol yields 0.425 Gm. (84%) of product, m.p. 64–66°. On recrystallization, the melting point is raised to 71.0 to 71.5° [reported, m.p. 72° (8)].

Preparation of 3-Ethyl-2-penten-4-ynal.—Stir a solution of freshly distilled ethchlorvynol (b.p. 72–73°/10 mm.) in 15 ml. of acid reagent (prepared by mixing 25 ml. of water, 20 ml. of sulfuric acid, and 50 ml. of ethanol) under nitrogen at 40° for 5 minutes. A yellow oil separates after about 3 minutes. Continue stirring at room temperature for 15 minutes, whereafter the mixture has become deep red. Pour into 40 ml. of ice-water and extract with petroleum ether (30–60°). Wash the extract with 5% sodium bicarbonate solution, dry over anhydrous magnesium sulfate, and evaporate under reduced pressure. Distillation of the residual oil under reduced pressure yields 2.62 Gm. (88%) of a pale yellow liquid, b.p. 48.5 to 49.5°/7 mm., η_D^{25} = 1.4851, which darkens on exposure to light and air.

The infrared spectrum of the compound in carbon tetrachloride shows absorption peaks at 3300 cm.$^{-1}$ (≡CH), 2740 cm.$^{-1}$ (—CHO), 2100 cm.$^{-1}$ (—C≡C—), 1675 cm.$^{-1}$ (unsaturated carbonyl), and 1600 cm.$^{-1}$ (conjugated double bond). The ultraviolet spectrum in ethanol shows broad absorption in the 235–260 mμ region ($\epsilon \sim 5500$) with $\lambda_{max.}$ at 240 mμ, ϵ = 5900.

Treatment of the above product with 2,4-dinitrophenylhydrazine in acidic (HCl) alcohol yields a derivative identical to that obtained directly from ethchlorvynol.

Anal.—Calcd. for $C_{13}H_{12}N_4O_4$: C, 54.14; H, 4.20; N, 19.43. Found: C, 54.59; H, 4.26; N, 18.87.

Treatment of the product with semicarbazide in aqueous alcohol yields a semicarbazone derivative, m.p. 179.5° dec.

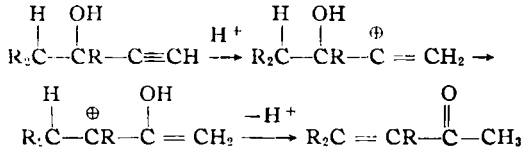
Anal.—Calcd. for $C_8H_{11}N_3O$: C, 58.16; H, 6.71; N, 25.43. Found: C, 57.80; H, 6.90; N, 25.61.

DISCUSSION

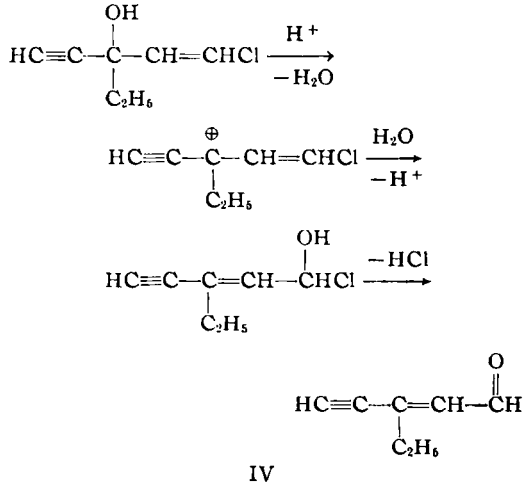
The acid catalyzed rearrangement of *tertiary*-acetylenic carbinols to α,β-unsaturated ketones is known as the Rupe reaction. The reaction appears to proceed either by a dehydration-hydration mechanism or by a straight acid-catalyzed rearrangement, depending upon reaction conditions (10–12).

TABLE II.—HOMOLOGS OF 2-PENTEN-4-YNAL

	$HC\equiv C-C(=O)-CH=CH-CH_2$ (14)	$HC\equiv C-C(=O)-CH=CH-CH_3$ (13)	$HC\equiv C-C(=O)-CH=CH-C_2H_5$
M.p. or b.p.	m.p. 19°	b.p. 53–54°/20 mm.	b.p. 48–49°/7 mm.
U. V. Spectrum $\lambda_{max.}$ (mμ); ϵ (EtOH)	257.5 18,000	261.5 12,500	240 5,900
2,4-Dinitrophenylhydrazone, m.p.	150° dec.	145°	125°
U. V. Spectrum $\lambda_{max.}$ (mμ); ϵ	...	385 33,500	375 32,800
Semicarbazone, m.p.	...	180° dec.	179° dec.
U. V. Spectrum $\lambda_{max.}$ (mμ); ϵ	...	292 32,500	289 31,000



Using an acidic 2,4-dinitrophenylhydrazine reagent (9), rearrangement and derivative formation occurs in one step. Ethinamate and methylparafynol react as expected to yield a derivative of the corresponding α,β-unsaturated methyl ketone. With ethchlorvynol, however, rearrangement to a carbonyl compound seems to proceed through an allylic 1,3-shift of the hydroxyl group with subsequent loss of hydrogen chloride leaving the acetylenic group intact



Analytical and spectral data are in agreement with structure IV. The mode of formation of the compound is analogous to that of 3-methyl-2-penten-4-ynal from 3-methyl-1-chloro-1-penten-4-yn-3-ol by allylic rearrangement (13). The properties of compound IV are compared with those of its homologs in Table II.

Using *p*-nitrobenzoic acid and *p*-toluenesulfonyl chloride in pyridine (8, 15), the tertiary alcohols are readily esterified to form the corresponding derivatives in excellent yield. With ethchlorvynol, yields of about 85% are obtained after heating the reaction solution on a steam bath for 40 minutes or more. Shorter reaction times of 10, 20, and 30 minutes give crude yields of 34, 52, and 72%, respectively.

The above procedures were used successfully for the identification of ethinamate and ethchlorvynol in pharmaceutical preparations. Ethinamate can be readily isolated from tablets by chloroform extraction, removal of the solvent, and crystallization of the residue from petroleum ether. Identification is then made by melting point determination and infrared analysis and confirmed by preparation of the 2,4-dinitrophenylhydrazone derivative. Ethchlorvynol is identified by dissolving the contents of one capsule in a small amount of alcohol and treating with 2,4-dinitrophenylhydrazine reagent. Alternatively, the contents of one capsule may be dissolved in chloroform, the chloroform solution washed with water, dried with anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue readily forms the *p*-nitrobenzoate derivative.

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Communications

Free 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one in Maize

Sir:

Since the discovery that methoxybenzoxazolinone (MBOA) is formed during the extractive treatment of wheat or maize plants (1) from 2,4-dihydroxy - 7 - methoxy - 1,4 - benzoxazin - 3 - one (aglucone), which is enzymatically released from its glucoside when the fresh plant tissue is disintegrated, the presence or absence of MBOA in uninjured maize tissue has been a matter of controversy between Beck *et al.* (2) and ourselves. We found no MBOA when the enzymes of the maize seedlings (Early Albert and a sugar maize variety, grown in a greenhouse) were destroyed by immersing the intact plants in boiling water or in cold alcohol (1). Only small amounts of free aglucone were present in 1 to 2-month-old plants, and free aglucone could not be detected in young seedlings.

The large amounts of MBOA found by Beck *et al.* (2) would imply a qualitative difference in