1225 w, 1250 w, 1265 m, 1350 w, 1370 w, 1400 m, 1450 m, 1500 m, 1530 s, 1580 w, 1620 m, 1690 s, 1745 s, 1800 m, 1850 s, 1980 w, 3230 m.

Anal. Calcd. for $C_{21}H_{31}NO_4$: C, 69.77; H, 8.65. Found: C, 69.76; H, 8.64.

Acknowledgment.—We are indebted to the Research Corporation for the partial financial support of this work.

Improved Synthesis of Oxotremorine

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Received January 21, 1965

Tremorine (1,4-dipyrrolidin-1-ylbut-2-yne) has been widely employed in the search for agents active against Parkinson's disease in man. The generalized tremor and spasticity caused in laboratory animals by tremorine is antagonized by drugs that are effective in the treatment of Parkinson's disease.[1,2] It has recently been suggested[3] that the pharmacological actions of tremorine may be entirely due to oxotremorine, an active metabolite[4] which Cho, *et al.*, isolated, identified as 1-(2-oxopyrroldin-1-yl)-4-pyrrolidin-1'-ylbut-2-yne, and synthesized.[5] Leslie and Maxwell[3] found that whereas some compounds of no clinical value in the treatment of Parkinson's disease were tremorine antagonists, anti-Parkinson drugs antagonized the actions of both tremorine and oxotremorine. The former compounds were presumed to have inhibited the oxidation of tremorine to oxotremorine which clearly has no bearing on central antitremor activity. These findings indicate that antagonism to oxotremorine should be a more discriminating test for anti-Parkinson agents, and a satisfactory practical source of oxotremorine would therefore seem to be of value.

Attempts to repeat the published synthesis[5] led to erratic results and the over-all yield of about 6% could not be duplicated. However, by conducting the reaction between pyrrolidone and propargyl chloride in liquid ammonia with sodamide as the condensing agent, N-propargyl-2-pyrrolidone was obtained in 83% yield. A Mannich reaction between this intermediate, formaldehyde, and pyrrolidine was carried out under the conditions described by Halsall and Thomas[6] for the preparation of 6-diethylaminohex-4-yn-1-ol. This provided a 61% yield of oxotremorine.

Experimental

N-Propargyl-2-pyrrolidone.—Sodamide was prepared from sodium (51 g., 2.2 g.-atoms) in about 2000 ml. of liquid NH_3. Pyrrolidone (170 g., 2.0 moles) was added dropwise to the stirred suspension. One hour later, 163 g. (2.2 moles) of propargyl chloride was added dropwise and stirring was continued a further 5 hr. After the NH_3 was allowed to evaporate overnight, the residue was stirred with ether and filtered (under N_2 to minimize fire hazard). Evaporation of the ether *in vacuo* and distillation of the residual oil gave the product as an almost colorless liquid: 204.7 g.; 83%; b.p. 76–86° (0.3 mm.); λ_{max}^{film} 3.13 (C≡C–H), 4.74 (C≡C), 5.92 μ (C=O).

1-(2-Oxopyrrolidin-1-yl)-4-pyrrolidin-1'-ylbut-2-yne (Oxotremorine).—A mixture of N-propargyl-2-pyrrolidone (12.3 g., 0.1 mole), 10 ml. of water, 7.4 g. (0.105 mole) of pyrrolidine, 6.3 g. (0.105 mole) of acetic acid, 8.5 g. (0.105 mole) of 37% aqueous formaldehyde solution, and 0.25 g. of cuprous chloride was stirred under nitrogen at 38–40° for 15 hr. The mixture was then extracted with ether followed by chloroform, and the combined extracts were dried and evaporated *in vacuo*. Distillation of the residue under reduced pressure and collection of the fraction boiling at 129–131° (0.1 mm.) provided 12.6 g. (61%) of oxotremorine. Pharmacological activity: maximal peripheral and central effects were observed in mice at a dose of 0.1 mg./kg. intraperitoneally.

Anal. Calcd. for $C_{12}H_{18}N_2O$: C, 69.87; H, 8.80; N, 13.58. Found: C, 69.70; H, 8.66; N, 13.24.

Acknowledgment.—The author is indebted to Dr. Meier E. Freed for valuable advice and encouragement.

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Benzylidene Derivatives of Indene and Cyclopentadiene[1,2]

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Received January 19, 1965

In the search for compounds which would have the antitumor activity of 4-(4-dimethylaminostyryl)quinoline without its toxicity to normal animals,[3] we have synthesized the series of benzylidene derivatives of indene and of cyclopentadiene listed in Table I. Haddow, *et al.*,[4] have reported antitumor activity of 9-(4-dimethylaminobenzylidene)fluorene and the greater activity of 4-dimethylaminostilbene. The stilbene structure is a part of the benzylidene derivatives of fluorene and indene but not of cyclopentadiene. It is interesting that none of the cyclopentadiene derivatives reported here showed strong antitumor effects, but several indene derivatives did. The minimum single i.p. dose required for clear-cut effect against Walker 256 tumors was about 40 mg./kg. for the NH_2, $NHCH_3$, and $N(CH_3)_2$ compounds, but the maximum tolerated dose was more than 15 times as large for the $N(CH_3)_2$ compound as for the other two. Lengthening the alkyl groups on the nitrogen increased the minimum effective dose. The presence of a CH_3 at the 3-position on the benzylidene group did not change the minimum effective antitumor dose, but lowered the maximum tolerated dose. A CH_3 group on the 3-position of the indene ring lowered the maximum tolerated

(1) This investigation was supported by Public Health Service Research Grants No. CA 03717-05 from the National Cancer Institute.

(2) Presented in part at the Southeastern Regional Meeting of the American Chemical Society, Charleston, W. Va., Oct. 16, 1964.

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(5) Under similar conditions this was also the minimum effective dose level of 4,4-dimethylaminostyrylquinoline.

TABLE I

INDENE AND CYCLOPENTADIENE DERIVATIVES

Compd.	Yield, %	M.p., °C.[a]	Formula	Calcd., % C	Calcd., % H	Found, % C	Found, % H	In methanol λ_{max}, mμ	In methanol log ε	In acetic acid λ_{max}, mμ	In acetic acid log ε	KB cell test[b] ED_{50}, γ/ml.	Tumor wt.[c] T/C @ mg./kg.		Killed @ mg./kg.	
1-(R-Benzylidene)indene																
4-NH_2	65	125[d-f]	$C_{16}H_{13}N$	87.94	5.98	87.90	6.12	385	4.4	370	4.2	13	0.0	80	1/2	125
													0.6	40	2/3	160
4-$NHCOCH_3$	31	188[f]	$C_{18}H_{15}NO$	82.73	5.79	82.45	5.83	360	4.5	360	4.6	20	0.8	40	1/3	80
4-$NHCH_3$	73	100–102[e]	$C_{17}H_{15}N$	87.15	6.02	87.42	6.44	405	4.5	400	4.2	25	0	80	1/3	80
													0.5	40		
4-$N(CH_3)_2$[g]	60	162[h]	$C_{18}H_{17}N$	87.44	6.93	87.28	6.74	420	4.6	340	4.3	>100	0	1600	3/3	2000[i]
										420	3.9		0	100	0/3	1600
													0.25	50		
4-NHC_2H_5	26	106–107[e,j,k]	$C_{18}H_{17}N$	87.44	6.93	87.70	7.03					27				
4-$N(C_2H_5)_2$	40	88–89.5[f]	$C_{20}H_{21}N$	87.22	7.69	87.54	7.34					31	0	640	0/3	1280
													0.12	320		
4-NHC_4H_9	43	94–95.5[e]	$C_{20}H_{21}N$	87.22	7.69	87.28	7.68	410	4.4	400	4.4	100				
4-$N(C_4H_9)_2$	46	62–63[j,l]	$C_{24}H_{29}N$	86.96	8.82	86.53	8.73	420	4.6	340	4.1	26	0.8	1600	0/3	1600
										420	4.2					
4-$NHCH_3$, 3-CH_3O	30	110–111[e]	$C_{18}H_{17}NO$	82.10	6.51[m]	82.00	6.59	415	4.5	405	4.3	33				
4-$N(CH_3)_2$, 3-CH_3O		89–91[e]	$C_{19}H_{19}NO$	82.28	6.91[n]	82.66	7.05	380	4.3	345	4.3	36				
4-$N(CH_3)_2$, 3-CH_3	30	93–94[f]	$C_{19}H_{19}N$	87.31	7.33	87.64	7.12	375	4.4	345	4.4	>200	0	320	2/2	625
													0.25	40		
4-$(CH_3)_2N$, 2,5-$(CH_3O)_2$	75	92–93[e]	$C_{20}H_{21}NO_2$	78.14	6.89	77.90	6.83	400	4.5	336	4.3	6.8	0.6	640	1/3	1280
2-CH_3O	50	61.5–63[f]	$C_{17}H_{14}O$	87.05	6.02	87.17	6.00					15				
4-CH_3O	50	123–124[f]	$C_{17}H_{14}O$	87.15	6.02	87.11	5.91	360	4.5	360	4.4	26				
2,5-$(CH_3O)_2$	38	91.5–92.5[f]	$C_{18}H_{16}O_2$	81.79	6.10	81.50	6.11	370	4.0	370	4.3	3.0, 5.1				
3,4-$(CH_3O)_2$	58	118–119[f]	$C_{18}H_{16}O_2$	81.79	6.10	81.58	6.11	365	4.5	365	4.4					
3,4-(—OCH_2O—)	64	114–115[j]	$C_{17}H_{12}O_2$	82.24	4.87	81.96	5.08					110				
2,4,5-$(CH_3O)_3$	60	113.5–114.5[j]	$C_{19}H_{18}O_3$	77.53	6.16	77.47	6.39	385	4.3	385	4.3	25	0.8	1600	0/3	1600
3,4,5-$(CH_3O)_3$	32	149[j]	$C_{19}H_{18}O_3$	77.53	6.16	77.16	6.21	355	4.4	360	4.4	>100				
4-$N(CH_2CH_2Cl)_2$	24	90–92[o]	$C_{20}H_{19}Cl_2N$	69.76	5.56	69.85	5.69									
1-(R-Benzylidene)-3-methylindene																
4-$N(CH_3)_2$	36	112–113[j]	$C_{19}H_{19}N$	87.31	7.33	87.35	7.42						0.3	640	1/3	640[p]
1-(R-Benzylidene)cyclopentadiene																
4-$NHCH_3$	64	101–102[e,q]	$C_{13}H_{13}N$	85.20	7.15[r]	85.16	7.19	400	4.5			23	0.7	640	2/2	625
4-$N(CH_3)_2$	64	106–107[f,q]	$C_{14}H_{15}N$	85.23	7.66	84.49	7.54	405	4.5	405	3.8	63	0.7	1280	0/3	1280
4-$N(CH_3)_2$, 2,5-$(CH_3O)_2$	58	96–97[j,s]	$C_{16}H_{19}NO_2$	74.68	7.44	74.41	7.59	405	4.3	360	4.1	17	0.5	1280	0/3	1280
4-$N(CH_2CH_2Cl)_2$	78	92–93[f,s]	$C_{16}H_{17}Cl_2N$	65.33	5.82	65.33	5.89					69				
4-(4-Dimethylaminostyryl)quinoline[t]												3	0.13	50	3/3	75

[a] Corrected for thermometer stem exposure; determined with Thiele tube. [b] Results of the standard *in vitro* KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center at the University of Miami Cell Culture Laboratory and Southern Research Institute. [c] We are grateful to Professor Alexander Haddow, Mr. J. E. Everett, and Mr. B. C. V. Mitchley of the Chester Beatty Research Institute for data on toxicity and activity against the Walker 256 tumor in rats weighing 200–250 g. Each compound was administered as a single i.p. injection in Arachis oil on the day following tumor implantation or on the first day of the toxicity observation. Tumor bearing animals were sacrificed approximately 8 days later and the average weights of tumors in treated and untreated hosts are reported as the ratio *T/C*. [d] All compounds are yellow unless otherwise indicated. [e] Recrystallized from isohexane. [f] Recrystallized from methanol. [g] M. Schultz, *Arch. Pharm.*, **253**, 168 (1915); C. Courtot, *Ann. Chim.* (Rome), **4**, 58 (1915). [h] Recrystallized from acetone. [i] Killed all mice at 800 mg./kg.; *T/C* equals 0.35 at 400 mg./kg., 0.56 at 200 mg./kg. with S180. [j] Recrystallized from 95% ethanol. [k] Further purified by alumina column and by Florisil column. [l] Red-brown. [m] *Anal.* Calcd.: N, 5.32. Found: N, 5.33. [n] *Anal.* Calcd.: N, 5.05. Found: N, 4.92. [o] Recrystallized from isooctane. [p] Also caused anemia 3rd to 7th day. [q] Red-orange. [r] *Anal.* Calcd.: N, 7.64. Found: N, 7.47. [s] Red. [t] For comparison.

dose and raised the minimum effective dose; so did two CH_3O groups at the 2- and 5-positions on the benzylidene groups.

The low toxicity of the indene derivatives might have been predicted on the basis of the relatively high ED_{50} figures found in KB cell culture tests, but the antitumor activity would not have been. It is interesting to note that some alkoxybenzylidene derivatives of indene, without any amino group, were among the most active compounds in inhibiting cell culture growth.

The ultraviolet absorption spectra of the aminobenzylideneindenes and cyclopentadienes showed peaks in the 360–420-mμ region, usually near 400 mμ, in methanol, as did the styrylquinolines, but in most cases the peaks were reduced in wave length or intensity or both by acetic acid. The compounds without amino groups had about the same maxima in both solvents.

Experimental

In a typical preparation, a solution of 0.04 mole of *p*-dialkylaminobenzaldehyde and 0.045 mole of indene in 200 ml. of absolute ethanol was heated to boiling, then 50 ml. of a saturated solution of KOH in absolute ethanol was added and the mixture was refluxed 25 min. The product which crystallized on chilling the solution was recrystallized from acetone or other convenient solvent. In some instances it was necessary to add water and precipitate the product as an oil. When cyclopentadiene was used in place of indene, the reaction mixture was prepared at room temperature and allowed to stand 30 min. before heating.

Nuclear Magnetic Resonance Spectra of Phenothiazines. Chemical Shift Data[1]

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Received September 1, 1964

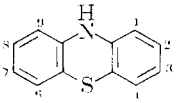
The widespread use of phenothiazine tranquilizers has given rise to extensive investigations into the structure of their metabolites,[3] variously thought to be hydroxylated in the 3-, 7-,[4] or 8-positions.[5] Since the infrared spectrum will not readily distinguish, *e.g.*, a 2,7-disubstituted phenothiazine from its 2,8-isomer[6] (both being 1,2,4-trisubstituted benzenes), and since the necessary synthetic hydroxylated phenothiazines are not yet available, the use of n.m.r. spectroscopy offered an attractive solution to the problem of unambiguous structure assignment of the metabolites of the phenothiazine drugs, if individual substitution sites in this molecule could be distinguished. This has now been shown to be feasible by an analysis of the n.m.r. spectra of the four possible monosubstituted compounds and of three types of disubstituted phenothiazines bearing a substituent in each benzene ring in the 2,7-, 2,8-, or 3,7-positions. In each case one substituent was a chloro or methoxy group. The preparation and n.m.r. spectra of 1,4- and 2,3-disubstituted compounds will be described in another paper.

Experimental

N.m.r. spectra were recorded using a Varian A-60 spectrometer. Chemical shifts are given in c.p.s. downfield from an internal tetramethylsilane standard. Approximately 5–10% solutions of the phenothiazines in perdeuteriodimethyl sulfoxide were prepared in a drybox under nitrogen. Dimethyl sulfoxide was found to inhibit autoxidation of the compounds. Melting points were taken in sealed evacuated capillary tubes on a Thomas–Hoover melting point apparatus, and are corrected. This method was found to give sharp melting points, frequently higher than those quoted in the literature, by suppressing the oxidation which inevitably occurred on using either a Kofler hot stage or an open capillary tube. Compounds were rigorously purified by repeated vacuum sublimation and were stored in sealed ampoules under nitrogen. A list of the substances[7] examined is given in Table I.

TABLE I

PHENOTHIAZINES USED TO OBTAIN CHEMICAL SHIFTS



Substitutional					
C-1	C-2	C-3	C-7	C-8	M.p., °C.
OMe					100.2–101.0
	OMe				184.0–184.5
		OMe			166.5–167.5
Cl					96.2–96.7
	Cl				202.8–203.2
		Cl			203–204
	CF_3		OMe		169–170
	CF_3			OMe	138.5–140
	CF_3			Cl	188.5–189
		OMe	OMe		201.0–202.5
	Cl		OMe		174.0–174.8
	Cl			Cl	269.5–270.5
	Cl		Cl		214.5–215.0
		Cl	Cl		239.5–240.0
Phenothiazine					184.0–184.5
10-Methylphenothiazine					102.5–103.0

Results

Comparison of the n.m.r. spectra of phenothiazine and 2-chlorophenothiazine on one hand, and those of the corresponding 10-(3-dimethylaminopropyl)substituted phenothiazines (promazine and chlorpromazine) on the other, showed clearly that the only difference between the spectra of each N-unsubstituted and N-substituted pair was the *bulk* chemical shift of all common hydrogen atoms. The individual chemical shifts of each aromatic hydrogen atom, and the coupling constants, were the same.

Analysis of the chemical shift data for phenothiazine and chlorine-substituted phenothiazines (in which the hydrogen frequencies are not altered by shielding[8])

(1) This investigation was supported by the National Institute of Mental Health, U. S. Public Health Service, through Research Grant MH-4582 and Contract PH 43-63-53.

(2) To whom inquiries should be addressed.

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