riety of *Nicotiana tabacum* which does not possess the inherited capacity to convert nicotine to nornicotine, it has been shown[8] that about 25% of the nicotine absorbed during culture disappears as such and does not reappear in the steam-volatile alkaloid fraction or in the ether extracts of alkalinized aqueous digests. Twenty seven per cent. of the absorbed *l*-nicotine and 13% of the *d,l*-nicotine failed of recovery in the present experiments as the sum of tertiary and secondary amine. Three and fourtenths per cent. of the *d,l*-N-ethylnornicotine, 48% of the *d,l*-N-methylanabasine and 26–27% of the *d,l*-N-ethylanabasine were lost. It is not known whether this loss has any connection with the dealkylation reaction with which the present study has been concerned.

Discussion

The remarkable non-specificity of the N′-dealkylation of these natural and synthetic compounds in the Nicotiana leaf suggests that the process may depend more upon some general property of the cell contents such as oxidation–reduction potential than upon questions of molecular geometry such as would be expected to occur, *e.g.*, between nicotine and a specific dealkylating or alkyl transferring enzyme. Our current objective is, therefore, the attempt to prepare extracts or leaf macerates which will exhibit dealkylating activity and which can be used to settle the question of enzyme participation.

N′-Ethyl alkaloids are not known to occur in Nicotiana, and their metabolism to nornicotine and anabasine in these experiments can be considered as indicative solely of the lack of specificity of the dealkylation process. In the case of N-methylanabasine, however, a possible biosynthetic link between this minor Nicotiana alkaloid and the parent alkaloid anabasine is indicated.

Acknowledgment.—The author wishes to thank the Misses Mary Elizabeth Eichrodt and Hope Howeth Robson for technical assistance. It is a pleasure to record the participation of Mrs. Cynthia James, colleague of Loftus Hills from Australia, who carried out the partition studies recorded herein. The work was aided variously by grants from the Dean's Special Fund and the Higgins Fund of Columbia and by substantial support from a large philanthropic foundation which prefers anonymity.

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(8) R. F. Dawson, *Arch. Biochem.*, **21**, 279 (1949); *Am. J. Bot.*, **27**, 190 (1940).

[CONTRIBUTION FROM ABBOTT LABORATORIES]

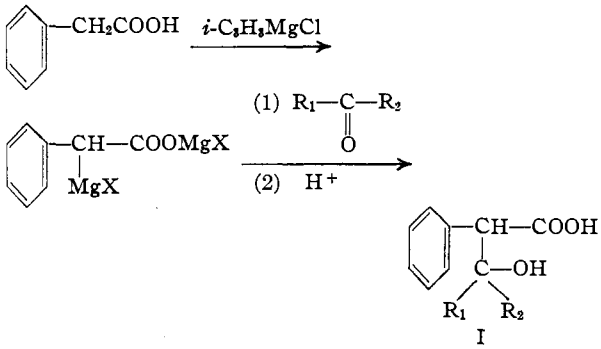
Antispasmodics. Esters of β-Alkyltropic Acids

BY ARTHUR W. WESTON AND ROBERT W. DENET

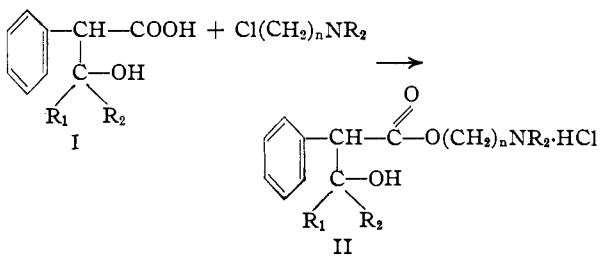
A series of β-alkyltropic acids was obtained by addition of the $C_6H_5CH(MgX)COOMgX$ or $C_6H_5CH(MgX)COONa$ Grignard complex to a number of aldehydes and ketones. Condensation of these acids with some basic alkyl halides in isopropyl alcohol solution gave the corresponding basic esters which have been found to possess antispasmodic action.

As part of a research program[1] directed toward the finding of a clinically useful antispasmodic, the investigation of a series of basic esters (II) derived from β-substituted tropic acids was undertaken.

The requisite acids (I) were obtained by treating phenylacetic acid or sodium phenylacetate with a slight excess of isopropylmagnesium halide and condensing the resulting Grignard complex with the appropriate aldehyde or ketone.[2] Phenylacetic acid is undoubtedly present as a contaminant in the reaction product and probably accounts for the difficulty experienced in purifying some of the simpler β-alkyl acids and the concurrent low yields. Although reaction with acetaldehyde and *n*-butyraldehyde introduced a second asymmetric carbon atom into the molecule, only one racemic modification was isolated in each case. Information pertaining to the acids prepared in this study is recorded in Table I.



The esters, listed in Table II, were prepared by condensing the acids and basic alkyl chlorides in boiling isopropyl alcohol solution.[3] Removal of the solvent, followed by addition of ether, was required to precipitate the basic ester hydrochlorides. Most of the products were obtained as oils which solidified after trituration with dry



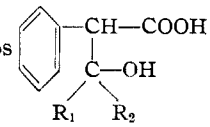
(1) For related papers see A. W. Weston, THIS JOURNAL, **68**, 2345 (1946); A. W. Weston and W. B. Brownell, in press.

(2) This reaction has been extensively investigated by Ivanov and co-workers; see D. Ivanov and N. I. Nicolov, *Bull. soc. chim. France*, [4] **51**, 1325 (1932), and previous references.

(3) H. Horenstein and H. Pählicke, *Ber.*, **71**, 1644 (1938),

TABLE I

β-ALKYLTROPIC ACIDS

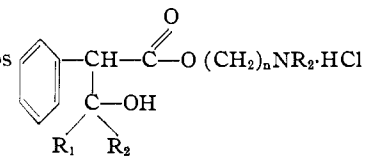


R_1	R_2	M.p., °C.	Yield, %	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
CH_3	H	133–134[a]	32	$C_{10}H_{12}O_3$	66.65	66.83	6.72	6.59
CH_3	CH_3	93–94[b]	66	$C_{11}H_{14}O_3$	68.02	68.21	7.26	7.50
n-C_3H_7	H	155–156[c,d]	37	$C_{12}H_{16}O_3$	69.20	69.00	7.74	7.74
C_2H_5	C_2H_5	154–155[e]	65	$C_{13}H_{18}O_3$	70.25	70.34	8.16	8.20
$-CH_2CH_2CH_2CH_2-$		96–97	82	$C_{13}H_{16}O_3$	70.89	71.21	7.32	7.54
$-CH_2CH_2CH_2CH_2CH_2-$		134–136[e,f]	85	$C_{14}H_{18}O_3$	71.70	71.90	7.74	7.58
n-C_3H_7	n-C_3H_7	176–177[g]	68	$C_{15}H_{22}O_3$	71.97	72.21	8.86	8.59

[a] Crystallized from water. [b] Crystallized from benzene. [c] Crystallized from ethyl acetate. [d] Reference 2 gives m.p. 155–156°. [e] Crystallized from dilute alcohol. [f] D. Ivanov and A. Spassov, *Bull. soc. chim. France*, [4] **49**, 377 (1931), report m.p. 135°. [g] Reference *f* records m.p. 171°.

TABLE II

BASIC ALKYL ESTERS OF β-ALKYLTROPIC ACIDS



R_1	R_2	n	NR_2	M.p., °C.	Yield, %	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
CH_3	H	2	$N(C_2H_5)_2$	69– 70.5[a]	[b]	$C_{16}H_{25}NO_3$	68.78	68.99	9.02	8.87	5.01	5.09
CH_3	CH_3	2	$N(C_2H_5)_2$	125–126.5[c]	52	$C_{17}H_{28}ClNO_3$	61.90	61.62	8.55	8.20	4.25	4.49
n-C_3H_7	H	2	$N(C_2H_5)_2$	117–118[c]	79	$C_{18}H_{30}ClNO_3$	62.72	62.95	8.79	8.77	4.07	4.25
C_2H_5	C_2H_5	2	$N(C_2H_5)_2$	119–120[c]	59	$C_{19}H_{32}ClNO_3$	63.76	63.57	9.01	8.85	3.91	3.71
$-CH_2CH_2CH_2CH_2-$		2	$N(C_2H_5)_2$	135–136[d]	67	$C_{19}H_{30}ClNO_3$	64.12	64.10	8.49	8.47	3.93	3.98
$-CH_2CH_2CH_2CH_2CH_2-$		2	$N(C_2H_5)_2$	138–139[e]	72	$C_{20}H_{32}ClNO_3$	64.93	65.19	8.72	8.47	3.78	3.71
$-CH_2CH_2CH_2CH_2-$		2	$N(CH_3)_2$	134–135[c]	56[f]	$C_{18}H_{28}ClNO_3$	63.24	63.52	8.25	8.26	4.09	4.25
$-CH_2CH_2CH_2CH_2CH_2-$		2	NC_4H_8O[h]	140–141[g]	[b]	$C_{20}H_{30}ClNO_4$	62.57	62.63	7.87	7.82	3.65	3.71
$-CH_2CH_2CH_2CH_2CH_2-$		3	$N(C_2H_5)_2$	135[i]	68	$C_{21}H_{34}ClNO_3$	65.59	65.96	8.92	8.83	3.64	3.52
n-C_3H_7	n-C_3H_7	2	$N(C_2H_5)_2$	128–129[c]	41	$C_{21}H_{36}ClNO_3$	65.35	65.54	9.40	9.56	3.63	3.62

[a] M.p. of free base from cyclohexane. [b] The yield was not determined since only a portion of the reaction product was worked up. [c] Crystallized from ethyl acetate. [d] Crystallized from isopropyl alcohol–ether. [e] Crystallized from isopropanol. [f] Crude yield. [g] Crystallized from ethanol–ether. [h] NC_4H_8O represents morpholino group. [i] Crystallized from absolute alcohol–ether.

ether. The hydrochlorides of diethylaminoethyl β-methyltropate and morpholinoethyl β,β-pentamethylenetropate (morpholinoethyl β-hydroxy-α-phenylcyclohexaneacetate) showed little tendency to crystallize and were converted to the free bases by treatment with carbonate solution. The base of the former compound solidified and was purified at this stage. In the latter instance, the base was reconverted to the hydrochloride salt which subsequently crystallized.

As an alternate approach to these esters, sodium β,β-pentamethylenetropate, produced by the interaction of sodium hydride and the free acid, was heated with diethylaminoethyl chloride in benzene. The only basic product isolated was diethylaminoethyl phenylacetate and the odor of cyclohexanone was present. Whether the reversal of the original addition occurred prior to or after ester formation has not yet been established. This tendency is increased if the tropic acid contains a β-aryl group. In several instances, when a β-aryltropic acid was refluxed with the basic alkyl halide in isopropyl alcohol solution only the corresponding phenylacetic ester resulted. It has been previously observed[4] that the β-substituted tropic acids revert quantitatively to phenylacetic acid and the carbonyl compound when heated in an aqueous alkaline solution.

Compounds of type II and their quaternary salts possess interesting pharmacological properties and preliminary results[5] indicate that these substances have a pronounced antispasmodic action.

Experimental

β,β-Tetramethylenetropic Acid (β-Hydroxy-α-phenylcyclopentaneacetic Acid).—A solution of 136 g. (1 mole) of phenylacetic acid in 1400 cc. of dry toluene was gradually added to the cooled Grignard reagent prepared from 384 g. (3.1 moles) of isopropyl bromide and 72 g. (3 moles) of magnesium metal in 900 cc. of ether. The mixture was allowed to stand at room temperature overnight. Following the removal of approximately 500 cc. of ether, a solution of 144 g. (1.2 moles) of cyclopentanone in 900 cc. of toluene was added to the Grignard complex. The reaction mixture was refluxed four hours, cooled and acidified with 1500 cc. of 15% sulfuric acid. The aqueous layer was separated, shaken with ether and the ether washings added to the original toluene layer which was then extracted with dilute sodium carbonate solution. Acidification of the combined alkaline extracts produced a sticky product which showed little tendency to crystallize. This material was taken up in ether and the ether solution was dried and concentrated. Trituration of the residue with Skellysolve B gave 198.5 g. (90%) of solid acid, m.p. 92–95°. Two crystallizations

(4) D. Ivanov and J. Popov, *Bull. soc. chim. France*, [4] **49**, 1547 (1931).

(5) The authors are grateful to Dr. R. K. Richards and members of the Pharmacological Department for this preliminary report of their findings.

from cyclohexane yielded 180 g. (82%) of product, m.p. 96–97°.

β,β-Di-*n*-propyltropic Acid (β-Hydroxy-α-phenyl-β-*n*-propylcaproic Acid).—To an ether solution of isopropylmagnesium bromide prepared in the usual manner from 217 g. (1.75 moles) of isopropyl bromide and 42.5 g. (1.75 moles) of magnesium metal, there was added 157.9 g. (1 mole) of sodium phenylacetate. The suspension was stirred and refluxed 48 hours. Then an ether solution of 200 g. (1.75 moles) of di-*n*-propyl ketone was added dropwise over a three-hour period. The reaction mixture containing the thick, semi-solid complex was stirred overnight, cooled and hydrolyzed by the gradual addition of excess dilute sulfuric acid whereupon partial separation of the product occurred. The solid thus formed was removed by filtration and subsequently dissolved in sodium carbonate solution. Acidification of the alkaline solution gave 173 g. of acid, m.p. 175–177°. Extraction of the ether layer of the original filtrate with carbonate solution and acidification of the basic extracts yielded another 67 g. of product, m.p. 168–170°. The two crops were combined and crystallized from dilute alcohol. There was thus obtained 170 g. (68%) of material, m.p. 176–177°.

Diethylaminoethyl β,β-Pentamethylenetropate Hydrochloride (Diethylaminoethyl β-Hydroxy-α-phenylcyclohexaneacetate Hydrochloride).—An isopropyl alcohol solution of 23.4 g. (0.1 mole) of β,β-pentamethylenetropic acid and 13.5 g. (0.1 mole) of diethylaminoethyl chloride was refluxed four hours. The solvent was removed at reduced pressure and the viscous residue triturated with dry ether. The product slowly solidified and was then collected by filtration and dried. This material weighed 35.8 g. (97%) and melted at 129–132°. Crystallization from an isopropyl alcohol–ether mixture gave 26.5 g. (72%) of product, m.p. 138–139°.

The methiodide salt was obtained by treatment of an ether solution of the free base with methyl iodide. The crystalline solid which separated melted at 138–138.5° after recrystallization from absolute alcohol. Prolonged boiling of the alcohol solution resulted in some decomposition of the product.

Anal. Calcd. for $C_{21}H_{34}INO_3$: C, 53.05; H, 7.20; N, 2.94. Found: C, 53.06; H, 7.07; N, 2.72.

Acknowledgment.—The authors wish to thank Mr. E. F. Shelberg, Head of the Microanalytical Department, and staff for the microanalyses reported in this paper.

NORTH CHICAGO, ILLINOIS RECEIVED MARCH 30, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE POLYTECHNIC INSTITUTE OF BROOKLYN AND THE DEPARTMENT OF BIOCHEMISTRY OF THE JEWISH HOSPITAL OF BROOKLYN]

A Study of α-Cholesterylene[1,2]

BY JOSEPH L. OWADES[3] AND ALBERT E. SOBEL

A new product of pyrolysis of a steroid sulfate was shown to be identical to the long known but uncharacterized α-cholesterylene. Based on absorption spectra, molecular weight determination, peracid titrations, selenium dehydrogenation, bromination, maleic anhydride addition, ozonolysis, color tests, thermal stability and failure to react with mercuric acetate, the structure 3,6′-bis-2,4-cholestadiene is proposed for this hydrocarbon.

Although the existence of α-cholesterylene has been known for over a hundred years, information on its structure and properties has been very meager. The authors became interested in the structure of α-cholesterylene during an investigation of steroid sulfates. In studying the pyrolysis of the aluminum salt of cholesteryl sulfate[4] (I), one of the products was found to be identical to the α-cholesterylene prepared by Zwenger in 1848[5] by the action of 80% sulfuric acid on cholesterol.

Zwenger's reaction may be described as a steroid color test, in which α-cholesterylene is an end-product. To the best of our knowledge, no end-product of a steroid color reaction has been characterized prior to the work reported here. For this reason, and because it had been postulated that steroid sulfates may be intermediates in sterol metabolism,[4] this study of α-cholesterylene was undertaken.

Past knowledge about α-cholesterylene consisted of a good ultimate analysis by Zwenger,[5] a molecular weight determination (cryoscopic in naphthalene) by Mauthner and Suida,[6] the optical rotation and an uncharacterized bromide by Eck,[7] and the melting point obtained by each of these investigators. The molecular weight and rotation are at variance with the results reported here.

In addition to the above work, two other reactions have been reported to yield α-cholesterylene: the action of zinc chloride[7] and of phosphorus pentoxide[8] on cholesterol. The identity of these products with α-cholesterylene was presumed on the basis of similar melting points. However, these reactions in our hands gave compounds that differed from α-cholesterylene in rotation (Table I), ultraviolet (Fig. 1) and infrared (Fig. 2) absorption spectra.

The structure of α-cholesterylene proposed as a result of this investigation (II) is based on the following evidence.

The unusually high melting point (290–300°) and its non-volatility at 270° at 0.2 μ pointed qualitatively to a compound of dimensions greater than C_{27}. The Rast determination presented some difficulties because of the low solubility and slow rate of dissolution of the steroid in molten camphor, but it finally yielded a molecular weight corresponding to a dimer. The steroid is not soluble in cyclopentadecanone (Exaltone).

The presence of the steroid nucleus was demonstrated by the isolation of 3′-methyl-1,2-cyclopen-

(1) Presented at the 117th Meeting of the American Chemical Society, Philadelphia, Penna., April, 1950.

(2) Abstracted from the dissertation submitted to the Faculty of the Graduate School of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(3) The Fleischmann Laboratories, Standard Brands, Inc., 810 Grand Concourse, New York 51, N. Y.

(4) A. E. Sobel, P. Owades and J. L. Owades, THIS JOURNAL, **71**, 1487 (1949).

(5) C. Zwenger, *Ann.*, **66**, 5 (1848).

(6) J. Mauthner and W. Suida, *Monatsh.*, **17**, 29 (1896).

(7) J. C. Eck and R. L. Van Peursem, *Iowa State Coll. J. of Science*, **13**, 115 (1939).

(8) T. Wagner-Jauregg, T. Lennartz and H. Kothny, *Ber.*, **74B** 1513 (1941).