Of all the proteins so far investigated, silk fibroin has the lowest leucine content (0.8%, determined by the solubility product method[8]), gelatin being next with 3.6%.

Summary

The *leucineless* strain of *Neurospora crassa* was used for the determination of leucine in hydrolysates of a number of proteins and of a few foodstuffs by the technique described previously.

Additional evidence for the reliability of the microbiological method has become available in the case of β-lactoglobulin, for which a leucine content of 15.7% has been found by Dr. G. L. Foster by the isotope dilution method as compared to 15.4% by the Neurospora method.

Ribonuclease is the only protein in which no leucine could be detected.

Of all the proteins so far investigated, silk fibroin has the lowest leucine content (0.8%), gelatin being next with only 3.6%.

Leucine accounts for an appreciable part of the molecule in zein (15.4%), bovine (13.7%), human (11.9%) and horse (13.0%) serum albumin, horse (15.7%) and human (14.7%) hemoglobin, insulin (13.4%) and β-lactoglobulin (15.6%).

Chymotrypsinogen, insulin and β-lactoglobulin contain 29, 45 and 50 residues of leucine, respectively, per mole of protein.

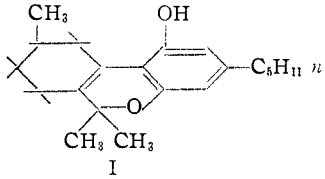
NEW YORK, N. Y. RECEIVED MAY 28, 1945

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS, AND THE PHARMACOLOGY DEPARTMENT, CORNELL UNIVERSITY MEDICAL COLLEGE]

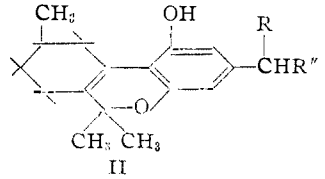
Tetrahydrocannabinol Homologs with a *s*-Alkyl Group in the 3-Position. XVI[1]

BY ROGER ADAMS, K. H. CHEN AND S. LOEWE

Modification of the synthetic tetrahydrocannabinol molecule (I) by changing the substituents in the 6- and 9-positions invariably produced compounds of lower marihuana potency. In the 3-position, however, an increase or decrease in the physiological activity could be attained with variation of the size of the alkyl group. Activity increased progressively from the methyl to the *n*-hexyl and then decreased in the higher homologs.[1,2,3]



I



II

With two exceptions the molecules previously studied had normal groups in the 3-position. Todd[3] described the isoamyl and isohexyl homologs and observed that they had only negligible activity by the Gayer test.

The investigation has now been extended to a series of molecules with alkyl groups containing a secondary carbon attached to the benzene ring (II). In these substances the physiological activity showed a marked increment in value over the corresponding normal alkyl derivatives. In Table I a comparison is given; all products were tested by the procedure previously described.

TABLE I

PHARMACOLOGICAL ACTIVITY OF TETRAHYDROCANNABINOL HOMOLOGS

	3-Substituent	No. of expts.	Potency
1	—C_4H_9-*n*	4	0.37 ± 0.12
2	—C_5H_{11}-*n*	20	1.00 standard
3	—$CH(CH_3)C_3H_7$-*n*	11	1.84 ± 0.13
4	—$CH(C_2H_5)C_3H_7$-*n*	11	1.67 ± 0.33
5	—$CH(CH_3)C_4H_9$-*n*	7	3.65 ± 0.33
6	—$CH(n\text{-}C_3H_7)C_4H_9$-*n*	8	3.17 ± 0.25
7	—C_6H_{13}-*n*	7	1.82 ± 0.18
8	—$CH(CH_3)C_5H_{11}$-*n*	13	4.85 ± 0.82
9	—C_7H_{15}-*n*	10	1.05 ± 0.15
10	—$CH(CH_3)C_6H_{13}$-*n*	10	16.4 ± 3.67
11	—C_8H_{17}-*n*	7	0.66 ± 0.12
12	Tetrahydrocannabinol[4] from cannabidiol (from American hemp)	20	7.3 ± 0.89
13	Natural tetrahydrocannabinol[5] acetate (from charas)	5	14.6 ± 1.05
14	Natural tetrahydrocannabinol[5] by hydrolysis of 13	15	7.8 ± 0.47
15	Pulegone + 5-(1-methylbutyl)-resorcinol	5	0.45 ± 0.05
16	Pulegone + 5-(*n*-amyl)-resorcinol	11	0.58 ± 0.12

Examination of Table I reveals several interesting facts. In all cases, the secondary groups

(1) For previous paper, see Adams, Loewe, Theobald and Smith, THIS JOURNAL, **64**, 2653 (1942).

(2) Adams, Loewe, Jelinek and Wolff, *ibid.*, **63**, 1971 (1941).

(3) Russell, Todd, Wilkinson, Macdonald and Woolfe, *J. Chem. Soc.*, 826 (1941).

(4) Adams, Loewe, Smith and McPhee, THIS JOURNAL, **64**, 694 (1942).

(5) Wollner, Matchett, Levioe and Loewe, *ibid.*, **64**, 26 (1942).

TABLE II

3,5-DIMETHOXYPHENYL OLEFINS

R = (3,5-Dimethoxyphenyl)	Yield, %	B. p., °C.	B. p., mm.	$n^{25}D$	Empirical formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H
2-R-pentene-2	79	124–129	2.3	1.5343	$C_{13}H_{18}O_2$	75.69	8.79	75.62	8.99
3-R-hexene-2(3)	80	136–140	4	1.5283	$C_{14}H_{20}O_2$	76.32	9.15	75.59	9.08
2-R-hexene-2	87	138–140	2.5	1.5301	$C_{14}H_{20}O_2$	76.32	9.15	76.35	9.25
4-R-octene-3(4)	..	150–153	3	1.5185	$C_{16}H_{24}O_2$	77.38	9.74	77.53	9.71
2-R-heptene-2-	87	149–159	3.5	1.5247	$C_{15}H_{22}O_2$	76.88	9.47	76.53	9.68
2-R-octene-2	77	163–169	4	1.5215	$C_{16}H_{24}O_2$	77.38	9.74	76.61	9.59

TABLE III

3,5-DIMETHOXYPHENYL ALKANES

R = (3,5-Dimethoxyphenyl)	Yield, %	B. p. °C.	B. p. mm.	$n^{25}D$	d^{25}_4	MR Calcd.	MR Found	Empirical formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H
2-R-*n*-pentane	83.5	114–119	2.3	1.5052	0.9837	61.94	62.60	$C_{13}H_{20}O_2$	74.96	9.68	75.09	9.86
3-R-*n*-hexane	76	123–126.5	2.2	1.5036	.9766	66.55	67.37	$C_{14}H_{22}O_2$	75.62	9.98	75.43	9.86
2-R-*n*-hexane	75	126–129	2.5	1.5021	.9707	66.55	67.59	$C_{14}H_{22}O_2$	75.62	9.98	75.96	9.99
2-R-*n*-heptane	75	135–137.5	3	1.4998	.9654	71.17	71.98	$C_{15}H_{24}O_2$	76.23	10.24	76.53	10.34
4-R-*n*-octane	..	137–142	2.5	1.4998	.9551	75.79	77.07	$C_{16}H_{26}O_2$	76.75	10.47	76.82	10.42
2-R-*n*-octane	81	150–153	3.5	1.4988	.9605	75.79	76.52	$C_{16}H_{26}O_2$	76.75	10.47	76.86	10.66

TABLE IV

5-*s*-ALKYL RESORCINOLS

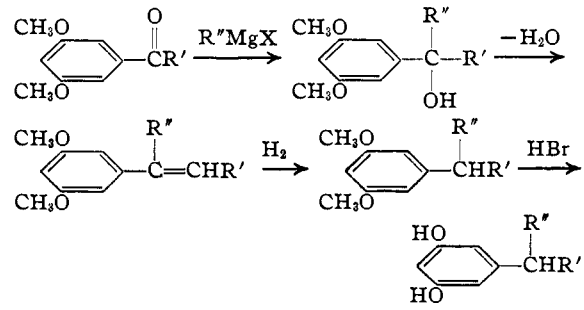
s-Alkyl group	Yield, %	B. p., °C.	B. p., mm.	Empirical formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H
1-Methylbutyl	70	161–165	3	$C_{11}H_{16}O_2$	73.30	8.95	73.40	8.98
1-Ethylbutyl	68	163–166	3	$C_{12}H_{18}O_2$	74.19	9.34	74.40	9.58
1-Methylpentyl	79	164–169	2	$C_{12}H_{18}O_2$	74.19	9.34	74.24	9.61
1-*n*-Propylpentyl	62	176–178	2.5	$C_{14}H_{22}O_2$	75.62	9.98	75.71	10.13
1-Methylhexyl	67	168.5–170	2	$C_{13}H_{20}O_2$	74.96	9.68	74.98	9.54
1-Methylheptyl	48	178–184	4	$C_{14}H_{22}O_2$	75.62	9.98	75.48	10.11

produce molecules more active than the isomeric normal groups (compare 2,3; 5,7; 8,9; 10,11). The increased activity is due primarily to the secondary group attached to the ring since the enlargement of one of the alkyls on the 1-carbon has an unimportant effect (compare 3,4; 5,6) apparently causing a slight decrease in the activity.

Unfortunately the investigation had to be temporarily suspended before additional molecules of this type with larger alkyl groups could be prepared. It is obvious that the maximum potency is to be found in molecules with secondary alkyl groups larger than the normal groups. The highest potency for a tetrahydrocannabinol prepared from cannabidiol from American hemp is 7.3 ± 0.89 as shown in the table (no. 12). Wollner, Matchett, Levine and Loewe[5] acetylated a purified red oil from charas and by careful fractionation under high vacuum obtained several fractions, the analysis of each of which coincided with that of tetrahydrocannabinol acetate and the most potent of which showed an activity of 14.6 ± 1.05 (no. 13). Hydrolysis of this compound, however, resulted in a tetrahydrocannabinol with an activity comparable with that obtained from cannabidiol (no. 14 and no. 12). The secondary octyl homolog (no. 10) is thus higher in potency, 16.4 ± 3.67, than any natural tetrahydrocannabinol or derivative ever isolated from a natural source. A further study of forked side chains in these molecules is contemplated.

The pulegone condensation product with 5-(1-methylbutyl)-resorcinol gave a pyran of low potency (no. 14) which was not significantly different from that of the *n*-amyl derivative (no. 15).

In preparing these homologs the method of Adams and Baker[6] was followed. The necessary 5-*s*-alkyl resorcinols were synthesized according to the scheme



The carbinols were dehydrated without purification to the corresponding olefins which were straw-colored liquids when one of the R's was methyl, but were colorless when the groups were larger. With Raney nickel as catalyst these olefins were hydrogenated smoothly at room

(6) Adams and Baker, THIS JOURNAL, **62**, 2405 (1940).

TABLE V

1-HYDROXY-3-R-9-METHYL-7,8,9,10-TETRAHYDRO-6-DIBENZOPYRONES

R	Yield, %	M. p. (cor.), °C.	Solvent for crystallization	Empirical formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H
1-Methylbutyl	70[a]	181 –181.5	Ethyl acetate	$C_{19}H_{24}O_3$	75.97	8.05	75.98	8.17
1-Ethylbutyl	73[a]	195.5–196	Ethyl acetate	$C_{20}H_{26}O_3$	76.40	8.33	76.22	8.53
1-Methylpentyl	53[b]	158 –159	Pet. ether (b. p. 60–110°)	$C_{20}H_{26}O_3$	76.40	8.33	76.40	8.34
1-*n*-Propylpentyl	51[b]	162.5–163.5	Ethyl acetate	$C_{22}H_{30}O_3$	77.15	8.83	77.05	8.96
1-Methylhexyl	47[b]	144.5–145	Ethyl acetate	$C_{21}H_{28}O_3$	76.80	8.59	76.87	8.38
1-Methylheptyl	62[a]	132.5–134	Ethyl acetate	$C_{22}H_{30}O_3$	77.15	8.83	77.10	9.12

[a] Condensation mixture allowed to stand at room temperature for about eighteen hours after an initial heating of five minutes. [b] Condensation mixture refluxed for five hours.

TABLE VI

1-HYDROXY-3-R-6,6,9-TRIMETHYL-7,8,9,10-TETRAHYDRO-6-DIBENZOPYRANS

R	%	B. p., °C.	B. p., mm.	Color	Empirical formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H
1-Methylbutyl	73	201–204.5	3	Brown	$C_{21}H_{30}O_2$	80.21	9.62	80.33	9.54
1-Ethylbutyl	76	211–213	3.5	Purple	$C_{22}H_{32}O_2$	80.43	9.82	80.29	9.77
1-Methylpentyl	70	173–181	1	Yellow	$C_{22}H_{32}O_2$	80.43	9.82	80.65	9.88
1-*n*-Propylpentyl	60	211–214	2	Yellow	$C_{24}H_{36}O_2$	80.85	10.18	80.80	10.05
1-Methylhexyl	74	208–213	2	Brown	$C_{23}H_{34}O_2$	80.65	10.01	80.70	10.24
1-Methylheptyl	56	217–222	2.5	Purple	$C_{24}H_{36}O_2$	80.85	10.18	81.15	10.41

temperature and under a pressure of two to three atmospheres, yielding colorless alkanes. Demethylation with hydrobromic acid resulted in the desired resorcinol derivatives which were yellowish oils. The yields and properties of these compounds are given in Tables II, III and IV.

Condensations of the 5-*s*-alkyl resorcinols with ethyl 5-methylcyclohexanone-2-carboxylate were effected with the aid of phosphorus oxychloride. Theoretically, two racemic pyrones are capable of existence in each case since two asymmetric carbon atoms are present. Only one, however, was ever isolated.

Experimental

The procedures used for the preparation of the pyrans and their intermediates are exemplified by the preparation of 1-hydroxy-3-(1-methylbutyl)-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran.

2-(3,5-Dimethoxyphenyl)-pentene-2.—To the Grignard reagent prepared from 2.45 g. (0.101 atom) of magnesium and 14.5 g. (0.101 mole) of methyl iodide in 60 cc. of ether, a solution of 21.1 g. (0.101 mole) of 3,5-dimethoxyphenyl *n*-propyl ketone in 40 cc. of ether was added. After refluxing for one hour the solution was thoroughly cooled and a saturated aqueous solution of ammonium chloride was added dropwise with stirring. When the decomposition was complete, the ether portion was poured out to be combined with the ether washings of the paste left in the flask. The ether solution was dried over calcium chloride, filtered and distilled to remove the solvent, leaving the carbinol behind. To dehydrate the latter it was distilled in the presence of a few drops of 20% sulfuric acid under moderately diminished pressure. Water began to be evolved at a temperature of 110–130°. The pure olefin was collected after all of the water had been expelled.

2-(3,5-Dimethoxyphenyl)-pentane.—The preceding pentene (15.9 g.) was hydrogenated at room temperature and under 2 to 3 atm. with Raney nickel as catalyst. Absolute ethanol was used as solvent. The reaction was completed in four hours. After removing the catalyst and the solvent, the product was distilled.

5-(1-Methylbutyl)-resorcinol.—A solution of 12.07 g. of 2-(3,5-dimethoxyphenyl)-pentane in 37 cc. of 48% hydrobromic acid and 111 cc. of glacial acetic acid was refluxed for five hours. The product was isolated and purified in the manner described by Suter and Weston.[7]

1-Hydroxy-3-(1-methylbutyl)-9-methyl-7,8,9,10-tetrahydro-6-dibenzopyrone.—A mixture of 5.9 g. (0.033 mole) of 5-(1-methylbutyl)-resorcinol, 6.1 g. (0.033 mole) of ethyl 5-methylcyclohexanone-2-carboxylate and 3.1 cc. (0.033 mole) of phosphorus oxychloride in 40 cc. of anhydrous benzene was heated to boiling for five minutes and then was allowed to stand for twenty-one hours at room temperature, while protected from moisture by a calcium chloride tube. The product was isolated in the usual manner and purified by recrystallization from ethyl acetate as colorless crystals.

1-Hydroxy-3-(1-methylbutyl)-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran.—This compound was prepared by the conventional method of treating the pyrone with excess (12 moles) of methylmagnesium iodide.

Condensation Product of Pulegone and 5-(1-Methylbutyl)-resorcinol.—Prepared from 1.42 g. of the resorcinol, 1.21 g. of pulegone and 0.3 cc. of phosphorus oxychloride according to the method of Adams, Smith and Loewe,[8] this compound boiled at 175–187° (2 mm.) (bath 215–230°). The golden yellow resin weighed 0.35 g.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 80.55; H, 10.03.

When 0.0489 g. was made up to 10 cc. with ethanol it gave α_D + 0.30°; *l*, 1; $[\alpha]^{29.5}_D$ + 61.35°.

Summary

A series of synthetic tetrahydrocannabinol derivatives, 1-hydroxy-3-alkyl-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyrans, has been prepared in which the 3-substituents are alkyl groups with a secondary carbon attached to the benzene ring. These have been compared with the corresponding compounds with normal alkyl groups in the same position and their marihuana potency was found to be much greater. In the

(7) Suter and Weston, THIS JOURNAL, **61**, 232 (1939).

(8) Adams, Smith and Loewe, *ibid.*, **63**, 1973 (1941).

molecules thus far prepared the presence of the 1-methylheptyl group had the greatest effect; the compound containing this group is more active than any of the known natural tetrahydrocannabinols.

URBANA, ILLINOIS RECEIVED JULY 9, 1945

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF ORGANIC CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, No. 303]

Condensations by Sodium. XXVIII. The Metalation of Sodium and Potassium Phenoxides

BY AVERY A. MORTON AND ROBERT L. LETSINGER

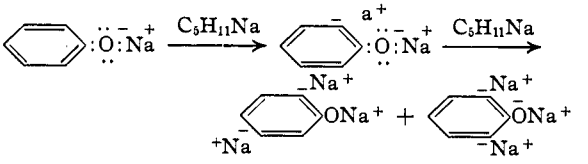
In a previous paper the metalation of benzylsodium by amylsodium was reported. Substitution of the second sodium atom was exclusively in the *meta* position. The orienting influence was attributed to the powerful electron-attracting sodium ion, adjacent to the anion.[1,2] In the present paper a study of the similar metalation of sodium and potassium phenoxide is reported. The object is to observe how much the substitution of an oxygen atom for a methylene group interferes with the *meta* directing influence of the alkali metal cation. The positions taken by the entering sodium ions were determined by carbonation and isolation of the acids.

Salicylic acid, 2-hydroxyisophthalic acid and hydroxyterephthalic acid were found. The first two products are the result of substitution in the *ortho* position but the last can result only from some *meta* substitution. The results of the study show, therefore, that the oxygen atom reduces but does not eliminate the *meta* directing influence of the alkali metal cation, when the conditions are such that the cation is adjacent to the anion.

Meta substitution in the phenoxide ion by an electrophilic reagent[2] is rare. Ingold[3] rates the *ortho–para* directing influence in the phenoxide ion as the most powerful known because the ion has a combined tautomeric and inductive effect. The easy preparations of tribromophenol and picric acid are examples. In such reactions the sodium phenoxide is in a dissociating solvent and the anion can act independently.

In the present experiments the solvent is the non-dissociating dodecane. Hence the phenoxide ion is adjacent to the alkali metal cation and is subject to the strong electron-attracting influence of that agent. The unshared electrons about the oxygen atom will dissipate much of this electron-attracting influence in somewhat the same way that the olefinic electrons screen out the electron-attracting influence of the nitro group in ω-nitrostyrene.[3]

Meta substitution is only partial. Moreover it is found only in the dimetalated product. Probably *ortho* metalation occurs first. The sodium ion attached to the ring can then exert a rear action on the electrons about the oxygen atom and thus weaken the screening effect which the electrons have on the other metal ion. The second substitution then takes place in either the *m*- or *o*-position, as shown.



The potassium ion adjacent to the phenoxide ion exerts a greater effect than does the sodium ion. The metalation by amylsodium is less complete even when the conditions are more strenuous, and the proportion of hydroxyterephthalic acid is 70% greater. These results are in excellent accord with the general behavior of *meta* directing agents in that the stronger the directing force, the slower the rate of substitution and the greater the percentage of *meta* product.

Experimental

Metalation of Sodium Phenoxide.—Phenol (6.2 g., 0.066 mole) was added rapidly at −7° to 15 g. of sodium sand suspended in 250 ml. of dodecane. The apparatus was the high speed stirring assembly used regularly in this work. *n*-Amyl chloride (20.2 g., 0.19 mole) in 30 ml. of dodecane was added dropwise to the cold mixture over a one hour period. The temperature was then raised over a two hour period to 50° at which point the color became gray instead of blue and the mixture became more viscous. The temperature was maintained at this level for one and a half hours, after which the mixture was allowed to cool and subsequently was carbonated. Water was added to remove excess sodium metal, the alkaline layer was extracted with ether, and the aqueous solution of the salts was acidified and then made basic with sodium carbonate. Ether extraction of this solution yielded 0.5 g. of phenol. Reacidification and extraction with petroleum ether and with benzene removed crude salicylic acid and traces of any caproic acid. Ether extraction then removed the mixture of dibasic acids.

The crude salicylic acid (0.6 g.) was purified by washing with petroleum ether and benzene and by recrystallizing successively from water, chloroform and water. When pure it melted at 157.5–159° (cor.) and showed no depression when mixed with an authentic sample.

The total dibasic acid amounted to 1.5 g. A portion (0.739 g.) of this mixture was separated into barium-soluble and -insoluble salts by addition of barium chloride to the ammonium salt solution. From the insoluble barium salt 0.51 g. of acid was isolated; from the soluble barium salt the amount obtained was 0.16 g. The former crystallized in long white needles which melted at 242°. The

(1) Morton, Little and Strong, THIS JOURNAL, **63**, 1339 (1943).
(2) Morton, *Chem. Rev.*, **35**, 1 (1944).
(3) Ingold, *Ann. Reports*, **23**, 129 (1926); **25**, 137 (1928).