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# CHEMICAL CONSTITUTION, PHYSIOLOGICAL ACTION AND PHYSICAL PROPERTIES IN A SERIES OF ALKYL PARA-AMINOBENZOATES

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The alkyl esters of *p*-aminobenzoic acid, because of their insolubility in water, cannot be employed for anesthetic purposes in aqueous solution, and the corresponding salts, though soluble, are far too acid in character to be used. The bases have, therefore, found their chief application as dusting powders. Numerous attempts have been made to correlate chemical constitution and physiological action of members of this series but there seems to be very little agreement among different investigators even where similar methods of testing were used; moreover, each previous worker has dealt with only a few members of the series. No attempt has been made to correlate physical properties and physiological action of these compounds.

This investigation involved the preparation and study of various alkyl *p*-aminobenzoates, namely, methyl, ethyl, propyl, *iso*propyl, *n*-, *iso*-, *sec.*- and *tert.*-butyl, *n*-amyl and allyl. The relative physiological action of these anesthetics on goldfish, the surface tension effects and the partition coefficients between amyl acetate and water have been determined and compared.

**Physiological Action.**—Sturmer and Luders[1] tested a few of these anesthetics on the mucous membrane of the mouth and found that the *n*-propyl ester was strong, *iso*propyl weaker, *iso*butyl weaker and that the *iso*amyl was strong but irritating. They concluded that increase in molecular weight of the alcohol results in decrease of anesthetic value and increase in toxicity.

Two Bayer patents[2] describe the comparison of the ethyl, *iso*propyl and *iso*butyl esters. In one-third saturated solution, the same effect was found for each as determined by tests on the cornea of a rabbit's eye. The conclusion was that saturated solutions of these three esters have the same effectiveness and that in equimolar solutions the *iso*butyl is twice as effective as the *iso*propyl and four times as effective as the ethyl ester.

T. Sollman[3] used a mixture of 10% anesthetic and 90% talcum powder and tested on the cornea of a rabbit's eye. He found the ethyl, *n*-propyl and *iso*butyl ester to have practically the same effectiveness. On the gums of human subjects Sollman with similar mixtures found the minimum con-

[1] Sturmer and Luders, *Deut. med. Wochschr.*, **34**, 2310 (1908).

[2] Bayer, Ger. pat. 211,801 (1909); 218,389 (1910).

[3] Sollman, *J. Pharmacol.*, **13**, 429 (1919).

centration of anesthetic which gives almost complete anesthesia: *iso*-butyl 2.5%, *n*-propyl 5.0–10%, ethyl 2.5–5%.

H. C. Brill[4] compared the *n*-butyl, allyl and *iso*propyl esters merely by placing the solid esters on the tongue, concluding that the three are equally effective.

It is obvious that the methods used by the previous investigators are very qualitative in character and a more quantitative method of comparison has been sought. This has resulted in the use of goldfish (*Carassius carassius*). These have previously been used for measuring the toxicity of digitalis extracts;[5] to determine the amount of pollution in waters;[6] and to determine the toxicity of various inorganic and organic materials.[7]

A few tests with solutions of these anesthetics showed that goldfish could be used for determining relative anesthetic values. After remaining in these solutions for a few minutes fish which to all appearances were dead and gave no evidence of irritation when their tails or fins were pressed with a stirring rod, revived after a certain period of time which varied from a few minutes to about an hour. These fish suffered no permanent ill effects because they were used again in other experiments (some of which involved the same anesthetic while others involved an entirely different one) and gave results consistent with those obtained with fish that had never been anesthetized before. Some fish were used four or five times and still gave consistent results at the end of that time. Usually an interval of about one week was allowed before they were used again. The advantage in this goldfish method of determining relative anesthetic values is that very dilute solutions may be used for the anesthetic determinations and that the experimental variations are not large.

The solutions of anesthetics were prepared by weighing out the exact amount of the finely powdered material and shaking in a volumetric flask with doubly-distilled water until solution was complete. For the less soluble members one to one and a half hours of continuous shaking was required. The solutions were allowed to stand overnight and were made up to volume the following morning.

Powers found that ordinary distilled water was toxic when it was prepared by distillation from copper stills and block tin leads. He found that fish would live from 30 to 52 days in water condensed in ordinary glass. All water used in this work was twice distilled from a Pyrex flask, the second time from alkaline potassium permanganate solution and condensed in glass. The middle two-thirds of the distillate was collected. The toxicity of the water prepared in the above way was tested by placing three fish in approximately 200 cc. of water contained in an 800cc. beaker. The

[4] Brill, THIS JOURNAL, **43**, 1320 (1921).

[5] Pittenger and Vanerkleed, *J. Am. Pharm. Assoc.*, **4**, 427 (1915); **8**, 893 (1919).

[6] Shelford, *Bull. Illinois State Lab. Nat. Hist.*, **13**, 125 (1918); **11**, 381 (1917).

[7] Powers, *Illinois Biol. Monographs IV*, No. 2 (1917).

water was changed every three or four days and the fish were fed on ordinary prepared flake fish food. One fish died in 51 days but the other two were perfectly healthy after 80 days and, to all appearances, were in as good condition as they were on the day they were placed in the water. These results indicate quite clearly that for all experimental purposes this doubly-distilled water may be regarded as entirely non-toxic.

In every case the tests were carried out at a temperature of 20° since Powers had found that the resistance of goldfish varies markedly with slight changes in the temperature. The fish were maintained in aquaria held at approximately this temperature and supplied with aerated tap water; the temperature was maintained by the admission of a small quantity of warm water and controlled by a thermostatic device. The fish were always maintained at exactly 20° for several hours preceding and during the actual experiments.

The effect of each concentration of anesthetic was tested by placing three fish (first carefully rinsed with distilled water) in 200 cc. of solution contained in an 800cc. beaker which was suspended in the constant-temperature aquarium. The first effect of this type of anesthetic is to stimulate respiration greatly so that rate of gill movement more than doubles; this was especially noticeable in the case of the *n*-butyl ester. After a few minutes the fish became quiescent and, after a time, usually turned very definitely on their sides with an apparent loss of sense of equilibrium. Shortly after, the fish became entirely motionless, resting on the bottom of the beaker. Their loss of feeling was tested by pressing their tails or fins with a glass rod. If anesthesia was not complete, this pressure caused a vigorous movement or twitching of their bodies; when anesthesia was complete no amount of pressure would cause the fish to move. When anesthesia was complete, these times were recorded as anesthetic time values. The fish were then rinsed with distilled water and placed in distilled water for recovery. During their recovery they would remain for a certain length of time in such condition that from all ordinary appearances they were dead. Then, depending on the concentration and also upon the anesthetic used, their mouths would begin to move and within a few minutes they would be prefectly normal again. The recovery of the fish treated with these anesthetics is practically 100%.

The weight of the fish (generally 1.5 g. to 4.5 g.) seemed to have no bearing on the variation in anesthesia time, since careful observation of hundreds of fish failed to bring out any orderly variation.

The method of comparing results was similar to that used by Powers for toxicity experiments with gold fish. The molar concentrations of an anesthetic solution were plotted as abscissas, the times for anesthesia as ordinates. With these points located, a curve can be drawn (see Fig. 1), the circles representing experimental data. The curves in all instances

represented within limits hyperbolas, the nature of which could better be illustrated by drawing the reciprocal curves. In these latter, the molar concentrations were plotted as abscissas, the reciprocals of the anesthetic times (multiplied by 100 to avoid fractions) as ordinates (see Fig. 1). This method of plotting gives straight lines within certain limits. At the lower end, the lines curved out and at the upper ends they curved over. The effects at the extremities of the lines were not studied, since this investigation is primarily concerned with comparative anesthetic values where they are normal and not complicated by various other factors. The higher

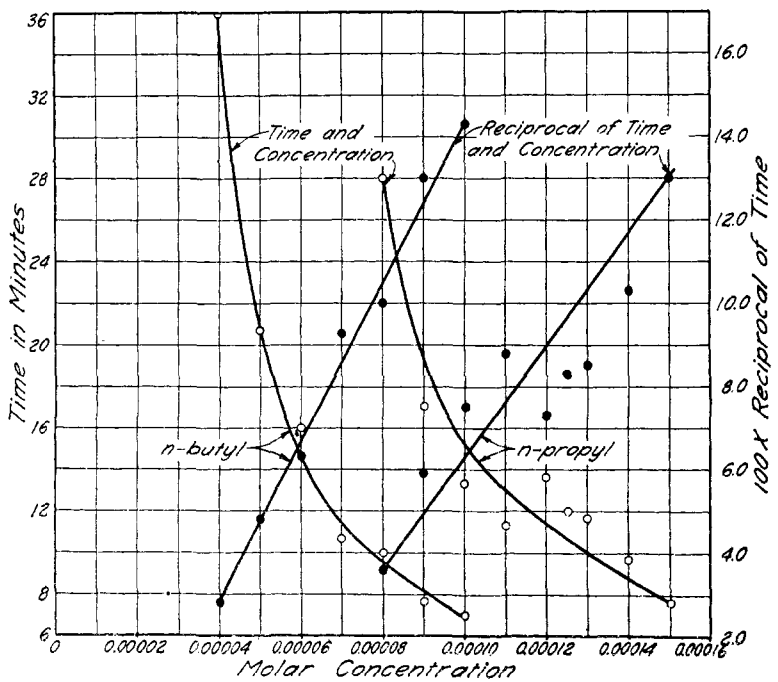


Fig. 1.

concentration of anesthetic causes abnormally rapid anesthesia, while very low concentrations may cause uncertain or incomplete anesthesia; as a consequence, for all these anesthetics, concentrations were, in general, chosen which yielded for anesthesia time a lower limit of about five minutes and an upper limit of about 40 minutes. In Table I are represented the experimental data obtained with *n*-propyl and *n*-butyl *p*-aminobenzoates and these data are plotted in Fig. 1 in the manner described above. These two anesthetics were selected because the data for the first were more irregular than for any of the other anesthetics while the data for the second were as regular as for any of the others. The data for the other eight anesthetics must be omitted on account of lack of space but they ap-

proached more nearly those of the *n*-butyl than of the *n*-propyl compound. In Fig. 2, however, are given the curves of all the anesthetics studied,

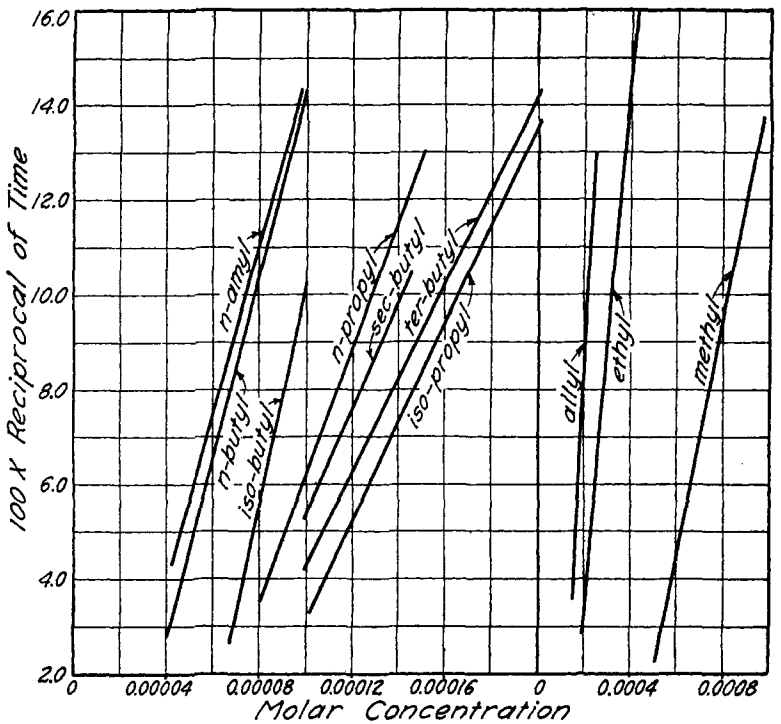


Fig. 2.

obtained by plotting from the experimental data the molar concentrations against the reciprocal of the times (multiplied by 100).

TABLE I

ANESTHESIA OF GOLDFISH WITH *n*-PROPYL AND *n*-BUTYL *p*-AMINOBENZOATES

| *n*-Propyl *p*-aminobenzoate | | | | *n*-Butyl *p*-aminobenzoate | | | |
|---|---|---|---|---|---|---|---|
| Molar concn. | Wt., g. fish | Anes. time | Av. | Molar concn. | Wt., g. fish | Anes. time | Av. |
| 0.00015 | 3 | 8 | | 0.0001 | 6 | 8 | |
| | 2 | 9 | | | 5 | 6 | |
| | 2 | 6 | 7.7 | | 3 | 7 | 7.0 |
| .00014 | 5 | 9 | | .00009 | 10 | 10 | |
| | 4 | 10 | | | 8 | 8 | |
| | 2 | 10 | 9.7 | | 3 | 5 | 7.7 |
| .00013 | 10 | 11 | | .00008 | 5 | 10 | |
| | 1.5 | 12 | | | 4 | 11 | |
| | 1.5 | 12 | 11.7 | | 4 | 9 | 10.0 |
| .000125 | 4 | 13 | | .00007 | 3 | 7 | |
| | 3 | 10 | | | 6 | 14 | |
| | 2 | 13 | 12.0 | | 3 | 11 | 10.7 |

TABLE I (*Concluded*)

| *n*-Propyl *p*-aminobenzoate | | | | *n*-Butyl *p*-aminobenzoate | | | |
|---|---|---|---|---|---|---|---|
| Molar concn. | Wt., g. fish | Anes. time | Av. | Molar concn. | Wt., g. fish | Anes. time | Av. |
| .00012 | 4 | 19 | | .00006 | 4 | 17 | |
| | 4 | 11 | | | 4 | 16 | |
| | 2 | 11 | 13.7 | | 3 | 15 | 16.0 |
| .00011 | 7 | 10 | | .00005 | 4 | 21 | |
| | 6 | 12 | | | 4 | 17 | |
| | 1.5 | 12 | 11.3 | | 4 | 24 | 20.7 |
| .00010 | 9 | 13 | | .00004 | 4 | 45 | |
| | 1.5 | 13 | | | 3 | 34 | |
| | 4 | 14 | 13.3 | | 2 | 30 | 36.3 |
| .00009 | 1.5 | 14 | | | | | |
| | 1.5 | 17 | | | | | |
| | 1.5 | 20 | 17.0 | | | | |
| .00008 | 5 | 29 | | | | | |
| | 3 | 25 | | | | | |
| | 3 | 30 | 28.0 | | | | |

TABLE II

COMPARISON OF VARIOUS VALUES FOR THE SERIES OF ANESTHETICS

| Ester | Molar concn. for anes. in 12.5 min. from exptl. curves in Table I | Anes. index | Threshold *M* concn. | Av. molar concn. | Amt. 100/*l* for each anes. |
|---|---|---|---|---|---|
| Methyl | 0.00074 | 0.4 | 0.00042 | 0.00075 | 8.5 |
| Ethyl | .00029 | 1.0 | .00013 | .000294 | 9.4 |
| Allyl | .000195 | 1.5 | .000128 | .000192 | 7.6 |
| *iso*Propyl | .000147 | 2.0 | .00004 | .000142 | 7.7 |
| *tert.*-Butyl | .000138 | 2.1 | .000062 | .000138 | 8.2 |
| *sec.*-Butyl | .000124 | 2.3 | .00006 | .000129 | 8.6 |
| *n*-Propyl | .000114 | 2.5 | .00004 | .000116 | 8.1 |
| *iso*Butyl | .000091 | 3.2 | .000052 | .0000835 | 6.4 |
| *n*-Butyl | .000068 | 4.3 | .00002 | .00007 | 8.2 |
| *n*-Amyl | .000063 | 4.6 | .000022 | .00007 | 9.3 |

With these lines available for each anesthetic, molar concentration of the various anesthetics which caused anesthesia in any given time were compared and the relative molar concentrations using the ethyl ester as a standard yielded anesthetic indices. In Table II, Col. 1 is given the molar concentrations which cause anesthesia in 12.5 minutes and in Col. 2 the resulting anesthetic indices. It is obvious from Chart 2, that because the lines are practically parallel, a comparison of molar concentrations at other times of anesthesia will result in essentially identical anesthetic indices.

It is of considerable interest that practically identical anesthetic indices may be calculated in a slightly different manner. If the hypothetical threshold concentration[8] for each anesthetic is determined (Table II,

[8] The hypothetical threshold concentrations designated as $\alpha$ are discussed by Oettingen, "Phanologie der Dorpater Lignosen," Dorpat (1879). An approximate

Col. 3) and a second point located for each anesthetic by taking the average of molar concentrations used and the average of the reciprocals of times of anesthesia (Table II, Cols. 4 and 5) these two points may be connected by lines and represent an approximate anesthesia time curve.[9] Anesthetic indices calculated from these lines at the average time and molar concentrations gave practically identical anesthetic indices.

The degree of accuracy of the experimental results was of interest. Complete data at different concentrations of the *n*-butyl compound were determined thrice. The anesthetic index thus calculated from each set of data varied only slightly and the variation was very much less than the difference between *n*-butyl and the next less effective anesthetic.

The difference between the *n*-butyl and *n*-amyl indices is so slight that no reliable conclusion may be drawn from these two indices.

During the anesthesia experiments, determinations of the recovery times were made, consisting in determining the time required for each fish to become normal after complete anesthesia and subsequent introduction into distilled water. The values were not regular and considerable variation occurred in fish used in the same solutions, perhaps due to the difficulty in removing entirely all the anesthetic solution from the gills. In general, it was noticeable that the recovery of the fish after anesthesia in dilute solutions of the anesthetics was much more consistent and much more rapid than in concentrated solutions. Moreover, in practically all the concentrations recorded with methyl, ethyl, allyl, *n*-propyl, *iso*propyl and *tert.*-butyl, recoveries were very rapid, being well within one to two minutes; in a few instances the higher concentrations of these anesthetics

threshold is determined by continuing what appears to be the straight line portion of the curve until it cuts the $X$ axis. The procedure is then a trial method in which values adjacent to the approximate threshold are substituted in the expression, $(X_1 - \alpha)\ t_1; (X_2 - \alpha)\ t_2; (X_3 - \alpha)\ t_3; \ldots\ldots (X_n - \alpha)\ t_n$, etc., where $X_1$, $X_2$ etc., represent the various concentrations of solution, $\alpha$ represents the hypothetical threshold, $t_1$, $t_2$, etc., represent the anesthesia times. That value which most nearly causes the sum of the absolute differences between the adjacent members of this series of expressions to approach zero is considered to be the hypothetical threshold. If the experimental values were such that they all fitted on the straight line, the absolute difference would be zero. In actual practice it is noticed that the values obtained for $(X_n - \alpha)\ t_n$ for points that are at the extremities of the straight line limits are considerably different from those that are obtained for points within the straight line limits. When this occurs, their values are discarded and the determination made with only those points that give the most consistent results.

[9] In the study of toxicity, many investigators have used for determining relative toxicity the expression $T$ (toxicity) $= \sqrt{\tan \Theta / \alpha}$ where $\Theta$ is the angle which the straight line portion of the curve makes with the $X$ axis and $\alpha$ is the hypothetical threshold of toxicity. Since anesthesia has been determined in a similar manner to toxicity, this expression might hold. However, there is considerable dispute about its value and the method adopted in this paper seemed a more reliable one for getting anesthetic indices. See also Sanderson and Peairs, *New Hampshire Agr. Expt. Sta. Bull.*, **7**, 11 (1913).

caused recovery to require five to six minutes. With the *iso*butyl and *sec.*-butyl, the recovery time for the lower concentrations ran from three to six minutes and, for the highest concentrations, from 10 to 15 minutes; with the *n*-butyl and *n*-amyl a much longer time was required except for the very lowest concentrations, the time of recovery varying from 10 to 60 minutes depending on the concentrations. Longer times were required for recovery with the *n*-amyl than with the *n*-butyl compound.

An attempt was also made to determine toxicity. Ten to twelve fish were placed in a concentration of anesthetic which was about the average of those concentrations used in determining anesthesia. The anesthesia time was recorded in the usual way and from that time on, the fish were removed at periodic intervals of about 30 minutes and introduced into distilled water. The longest times that the fish could remain in the anesthetic solutions and still recover, it was hoped, would represent in a rough way the relative toxicities. Unfortunately, many other factors were obviously coming into play which affected the results, and the toxicity experiments were not carried out at more than one concentration. The maximum time the fish could remain in the solutions varied from 180 to 400 minutes depending upon the anesthetic used, and the actual concentration of the anesthetic solution seemed to be an important factor. It was particularly noticeable that fish could not remain as long in 0.00075 *M* methyl, 0.00025 *M* ethyl or 0.00015 *M* *tert.*-butyl solutions as they could in 0.000167 *M* *iso*propyl, 0.00015 *M* *sec.*-butyl, or 0.0001 *M* *iso*butyl solutions and in these they could remain less time than in 0.0002 *M* allyl, 0.000135 *M* *n*-propyl, or 0.000067 *M* *n*-butyl. Finally, they remained longest without fatal effects in 0.000075 *M* *n*-amyl solution.

From the anesthetic indices several conclusions can be drawn about structure and pharmacological action for equimolar concentrations. (a) An increase in the length of the carbon chain of the alkyl group increases the anesthetic effect until the *n*-butyl ester is reached. From then on, as concluded from the data on *n*-amyl, the effect will not be so great—probably due partly to marked insolubility of the esters. (b) The *n*-alkyl derivatives are much more effective than the isomeric branched alkyl derivatives. (c) The anesthetic power of the four isomeric butyls decreases in the order *n*-butyl, *iso*butyl, *sec.*-butyl, *tert.*-butyl. If the first three are considered as derivatives of the *n*-propyl ester with a methyl substitution on each of the three carbon atoms, it may be concluded that substitution on the carbon atom alpha to the oxygen decreases the anesthetic action, while substitution on the $\beta$-carbon increases the anesthetic action somewhat and on the $\gamma$-carbon atom increases it markedly. (d) The allyl ester lies between the *n*-propyl and ethyl and if any conclusion may be drawn from this single example, it is that unsaturation in the $\beta,\gamma$ position decreases the anesthetic action.

The goldfish experiments were carried out in the Zoölogical Laboratory of the University of Illinois with the helpful suggestions and advice of Dr. V. E. Shelford. The authors wish to express their thanks to Dr. Shelford for this aid.

**Surface Tension and Partition Coefficients.**—The anesthetic action of many substances may be associated with certain physical properties. Overton and Meyer[10] attributed a high lipoid-to-water partition ratio for active anesthetics. Again Overton, Löw and Ehrlich[11] formulated a list of "active groupings" in the order of anesthetic power. It may be observed that these groupings are of the type classed as polar and would lead to the conclusion that the anesthetic action might be associated with some change at a lipoid-water interface rather than in the interior of a lipoid material. At such an interface, a composite molecule of a lipoid-soluble tail and polar head would be strongly adsorbed, thus altering the surface energy of the interface. Whether narcosis is produced by this alteration in the interfacial surface tension alone, or whether it is the result of displacement of some other component of the surface is unknown. In many cases adsorption at interfaces such as at a benzene-water interface, is associated with adsorption at an air-water interface and the alteration in the surface tension of water produced by the addition of the anesthetic may be taken as an approximate measure of adsorption at a lipoid-water interface. In the case of the *p*-aminobenzoic esters, all of which contain identical polar groupings, the drop number as well as the partition coefficient between aqueous solutions and some polar solvent may be expected to give some information on the interfacial adsorption and thus on the anesthetic value.

The results of the surface tension and partition coefficients are given in Table III.

TABLE III

SURFACE TENSION EFFECT OF ANESTHETICS

| Ester | Molar concn. | Drop no. | $dy/dc$ | $K = \frac{C(CH_3CO_2C_5H_{11})}{C(H_2O)}$ |
|---|---|---|---|---|
| Methyl | 0.001 | 49.7 | 1.94 | .. |
| Ethyl | .001 | 51.7 | 4.41 | 317 |
| Allyl | .004 | 56.5 | 12.74 | 505 |
| *iso*Propyl | .001 | 51.7 | 17.27 | 646 |
| *n*-Propyl | .001 | 51.7 | 17.27 | 1470 |
| *tert.*-Butyl | .0005 | 54.0 | 62.41 | 1190 |
| *sec.*-Butyl | .0005 | 54.0 | 62.41 | 3820 |
| *iso*Butyl | .0005 | 56.7 | 95.79 | 5800 |
| *n*-Butyl | .0005 | 56.7 | 95.79 | 6010 |
| *n*-Amyl | .0001 | 51.3 | 128.40 | 25800 |

[10] Overton, "Studien über die Narkose, zugleich ein Beitrag zur Allgemeinen Physiologie," Jena, G. Fisher, 1901, p. 70.

[11] Ehrlich, "Konstitution, Verteilung und Wirkung chemischer Körper," Leipzig, 1893.

This table would indicate that increasing the length of the ester group tends to increase surface tension effect of the anesthetic. Apparently very little difference appears between the values for the *iso*propyl and *n*-propyl or *iso*butyl and *n*-butyl compounds, but the *sec.*-butyl and *tert.*-butyl give distinctly different results from the *n*-butyl. The allyl has less effect than the propyl compound.

The distribution ratio shows more or less regular change with increase in the size of the ester group. The value for the *n*-propyl is greater than for the *iso*propyl, those for the *n*-butyl and *iso*butyl are approximately the same, but both are larger than those for the *sec.*- or *tert.*-butyl. The value for the allyl is somewhat lower than that for the propyl compound.

Finally, a surprising agreement exists between the surface tension, the distribution coefficient and the anesthesia on goldfish as shown by the indices given in Table IV.

TABLE IV

COMPARISON OF INDICES FROM ANESTHESIA, SURFACE TENSION AND DISTRIBUTION COEFFICIENT

| Ester | Goldfish | Surface tension | Distribution coefficient |
|---|---|---|---|
| Methyl | 10 | 10 | 10 |
| Ethyl | 9 | 9 | 9 |
| Allyl | 8 | 8 | 8 |
| *iso*Propyl | 7 | 7(6) | 7 |
| *tert.*-Butyl | 6 | 5(4) | 6 |
| *sec.*-Butyl | 5 | 4(5) | 4 |
| *n*-Propyl | 4 | 6(7) | 5 |
| *iso*Butyl | 3 | 3(2) | 3 |
| *n*-Butyl | 2 | 2(3) | 2 |
| *n*-Amyl | 1 | 1 | 1 |

From this table it would seem clear that with this series of anesthetics the distribution ratio and in a general way the surface tension may be used as an indication of the anesthetic effect. In comparing any homologs in this series it is obvious that the distribution ratio will give more significant results since the difference in the value of the distribution ratio between homologs is larger and can be determined more accurately than the surface tension effect.

**Preparation of Esters.**—The various esters of *p*-amino benzoic acid were made by condensing *p*-nitrobenzoyl chloride with alcohols and subsequently reducing the nitro esters with iron or by catalysis with platinum oxide-platinum black. In general, the nitro esters are liquids and can be purified by distillation under diminished pressure. The amino esters are in every case solids, but many of them were most readily purified by distillation under diminished pressure and subsequent crystallization from benzene. The samples of ethyl and *n*-butyl *p*-aminobenzoate were

kindly donated by the Abbott Laboratories, North Chicago, Illinois. They were recrystallized twice before using in order to make certain that they were of the very highest purity.

**Nitro Esters.**—The general procedure for making those nitro esters in which a primary alcohol was used consisted merely in warming on a water-bath for several hours a mixture of an excess of alcohol with *p*-nitrobenzoyl chloride. The excess of alcohol was then distilled off and the nitro esters were purified by distillation under diminished pressure. The yields were practically quantitative.

In making the esters of secondary alcohols, a similar procedure can be used and, in general, the yields are very good. In the case of *tert.*-butyl alcohol, however, such a procedure results in the formation of large amounts of benzoic acid and *tert.*-butyl chloride. In order to obtain good results the following method is employed, a method which can also be used to advantage in the preparation of the esters of the secondary alcohols.

A mixture of one molecular equivalent of *p*-nitrobenzoyl chloride and one molecular equivalent of a secondary or tertiary alcohol is mixed with about an equal volume of benzene. The mixture is warmed in order to give a homogeneous solution, after which slightly more than one molecular equivalent of pyridine is added gradually with warming and shaking. The reaction takes place immediately upon the addition of the pyridine. A convenient method for the isolation of the ester is to add sufficient benzene to make the total reaction mixture homogeneous, providing it is not already so. This solution is then extracted several times with dil. hydrochloric acid to remove the pyridine, once or twice with sodium carbonate to remove traces of acid chloride or acid, and finally the benzene is distilled in order to obtain the nitro ester.

By this process, the yields of the pure esters vary from 75 to 90% of the calculated amount.

TABLE V

ESTERS OF *p*-NITRO BENZOIC ACID[a]

| | M. p. or b. p., °C., this research | Lit. | Other const. | Analysis Subs. | $N_2$, cc. | Calcd. % | Found % |
|---|---|---|---|---|---|---|---|
| *iso*Propyl | 108.5 (95% alc.) | 110–111[b] 95[c] | | | | | |
| *sec.*-Butyl | 25° | | | | | | |
| | 136–139 (2–3 mm.) | | $n_D^{26}$ 1.5170 | 0.3405 | 20 | 6.28 | 6.48 |
| | | | $d_{25}^{30}$ 1.1502 | | (27°, 751 mm.) | | |
| *tert.*-Butyl | 115.5 (95% alc.) | | | 0.2208 | 13.4 (32°, 749 mm.) | 6.28 | 6.50 |
| *n*-Amyl | 159–161 (4 mm.) | | $n_D^{22}$ 1.5188 | | | | |
| | | | $d_{20}^{20}$ 1.1410 | | | | |
| Allyl | 28.5[d] | | | | | | |
| | 165–168 (13 mm.) | 178 (19 mm.) | $n_D^{29}$ 1.5459 | | | | |
| | 127–128 (1–2 mm.) | | $d_{20}^{30}$ 1.2283 | | | | |

[a] Only those which are new or for which different constants have been found have been given in the table.

[b] Ger. pat. 211,801 (1908).

[c] Brill, THIS JOURNAL, **43**, 1320 (1921).

[d] U. S. pat. 1,360,994 (1921).

**Amino Esters.**—The nitro esters were reduced to amino esters by mixing with a large excess of iron powder and water to form a paste, adding a very small amount of hydrochloric acid (about 1 cc. of concd. acid to 25 g. of ester) and stirring until the reduction was complete. In the case of certain nitro esters such as the *iso*propyl, it is necessary to warm the mixture during the entire reduction. On the other hand, most of the nitro esters during the reduction must be cooled occasionally, particularly at the beginning and, in the case of the allyl ester, a slush of ice and water is necessary to prevent the water of the reaction mixture from boiling. After the mixture has been stirred until no more heat is evolved, it is advisable to heat on the water-bath for one to two hours longer, and stir from time to time. The amino esters are then extracted by means of benzene.

The nitro compounds except the allyl can also be reduced very conveniently by dissolving 0.1 mole in 150 cc. of alcohol, adding 0.2 g. of platinum oxide[12] and reducing with hydrogen at 2 to 3 atmospheres' pressure. The time required for reduction is about ten minutes.

The amino esters are generally most conveniently crystallized from benzene. It is found, however, that the compounds obtained by the iron reduction, as described above, are frequently slightly colored and this re-

TABLE VI

ESTERS OF *p*-AMINOBENZOIC ACID

| | M. p. or b. p., °C., this research | Lit. | Analysis Subs. | $N_2$ | Calcd. N, % | Found % |
|---|---|---|---|---|---|---|
| Methyl | 112 | 112[a] | | | | |
| Ethyl | 89 | 89[b] | | | | |
| *n*-Propyl | 73 | 73–74[c] | | | | |
| *iso*Propyl | 84 | 85–86;[d] 79[e] | | | | |
| *n*-Butyl | 57 | 59;[f] 58[e] | | | | |
| *iso*Butyl | 64 | 65[g] | | | | |
| *sec.*-Butyl | 55 | | 0.3632 | 26 cc. (34°, 748 mm.) | 7.25 | 7.61 |
| *tert.*-Butyl | 109.5 | | .2241 | 15.3 cc. (29°, 751 mm.) | 7.25 | 7.47 |
| *n*-Amyl | 53 | | .2757 | 17.6 cc. (30°, 749 mm.) | 6.76 | 6.95 |
| Allyl | 52.5 | 51–52[h] | | | | |

[a] (a) Alfred Einhorn, *Ann.*, **311**, 158 (1900). (b) John Johnston, *Proc. Roy. Soc.*, (London) **78A**, 82 (1906).

[b] Limpricht, *Ann.*, **303**, 278 (1897); **320**, 135 (1902). Vorländer and Meyer, *Ann.*, **320**, 135 (1902). H. Salkowski, *Ber.*, **28**, 1921 (1895).

[c] Ger. pat. 213,459 (1908).

[d] Ger. pat. 211,801 (1908).

[e] Brill, THIS JOURNAL, **43**, 1320 (1921).

[f] Brit. pat. 148,743; 153,827 (1920); *C. A.*, **13**, 1030 (1921); see also U. S. pat. 1,440,652 (1923).

[g] Ger. pat. 218,389; E. Impens, *Therap. Gegenwart*, **51**, 348 (1910).

[h] U. S. pat. 1,360,994 (1921); Ref. e.

[12] Adams and Shriner, THIS JOURNAL, **45**, 2171 (1923).

duction color can be removed only by several crystallizations. If, however, the amino ester is vacuum-distilled with a small amount of zinc dust, a distillate is obtained which, after one crystallization, is colorless and pure. All of the amino esters studied could be distilled without decomposition under a pressure of 2 to 6 mm. with the exception of the *tert.*-butyl. This substance, however, was quite readily purified by crystallization.

## Summary

1. A series of alkyl *p*-aminobenzoates consisting of the methyl, ethyl, *n*-propyl, *iso*propyl, *n*-butyl, *iso*butyl, *sec.*-butyl, *tert.*-butyl, *n*-amyl and allyl have been prepared.

2. The surface tension effects of these various anesthetics have been determined and their partition coefficients between amyl acetate and water.

3. The anesthetic effects of these substances on goldfish have been quantitatively determined and the results plotted.

4. A comparison of the anesthetic indices, partition coefficients and surface tension effects shows a striking parallelism.

5. From the anesthetic indices, it may be concluded that (a) increase in the length of the carbon chain of the alkyl group increases the anesthetic effect until the *n*-butyl is reached. From that point on, the increased effect will not be so great, probably due to marked insolubility of the esters with further increase in molecular weight; (b) the *n*-alkyl are more effective than the branched-chain alkyl derivatives.

URBANA, ILLINOIS

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

# ISOXAZOLINE OXIDES
# V. CARBOXYL DERIVATIVES

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Isoxazoline oxides that have carboxyl groups attached to the ring can be made by a series of reactions similar to those which have been employed for getting all isoxazoline oxides that are known. Thus phenylnitromethane combines with benzal-malonic ester and forms a substance which must be a γ-nitro ester, because it is also formed by the addition of malonic ester to nitrostilbene.

$$C_6H_5CH{:}C(CO_2C_2H_5)_2 + C_6H_5CH_2NO_2 \rightleftharpoons C_6H_5CHCH(CO_2C_2H_5)_2$$

$$C_6H_5CH{:}C(NO_2)C_6H_5 + CH_2(CO_2C_2H_5)_2 \qquad C_6H_5CHNO_2$$

The γ-nitro ester, when brominated, yields an α-bromo-γ-nitro ester and this in turn yields an isoxazoline oxide on elimination of hydrogen bromide.