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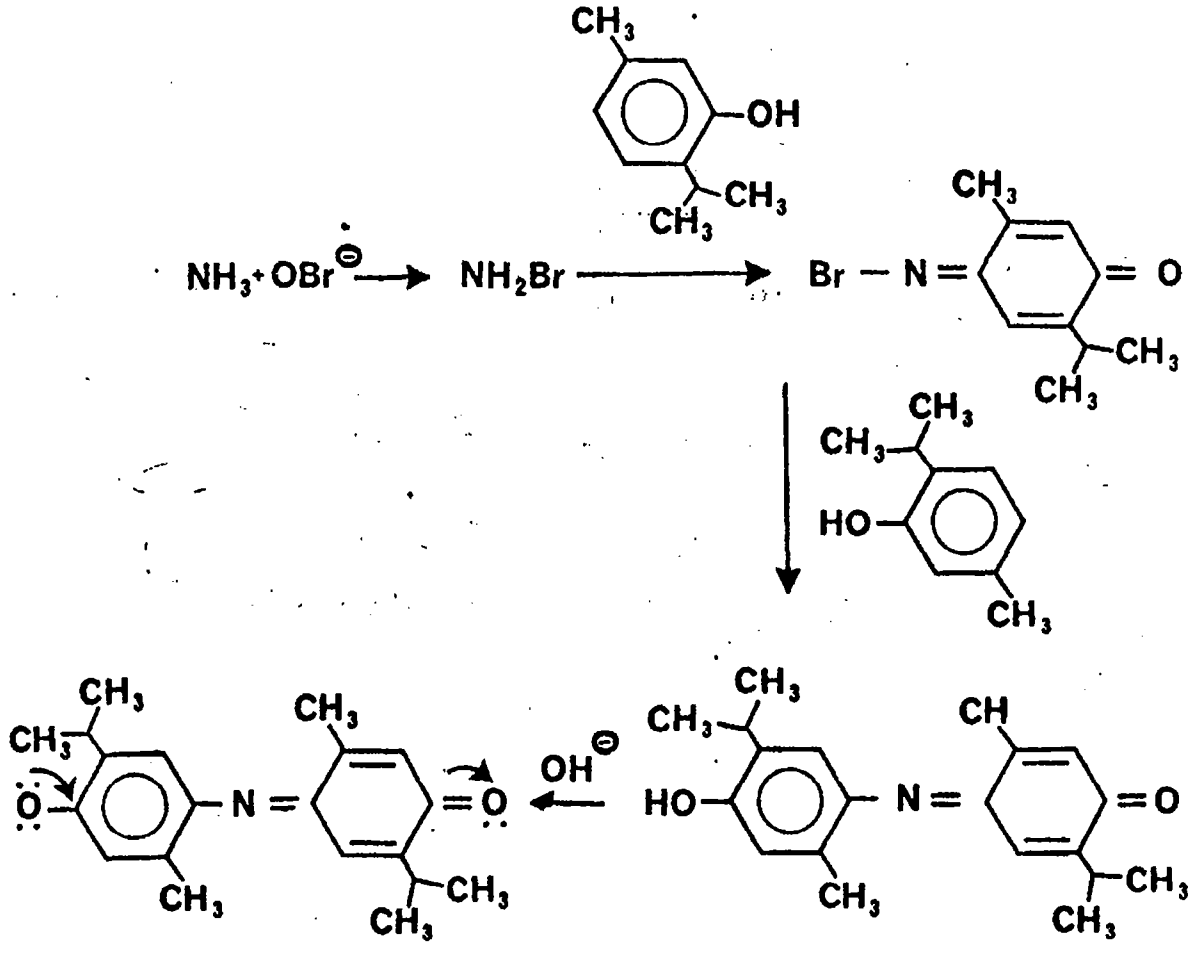
COLLABORATIVE STUDY OF THE DETERMINATION OF AMMONIA AS AN INDEX OF DECOMPOSITION IN CRABMEAT

By Kurt Steinbrecher, Seattle District

The study by Burnett (1) showed that a general correlation existed between the concentration of ammonia in crabmeat and its degree of decomposition by organoleptic standards. The method is based on the color formation observed after the reaction of ammonia, thymol, and alkaline bromine water. The reaction scheme may be suggested to be analogous to the indophenol mechanism proposed by Bolleter et al. (2):



Fernandez-Flores and Salwin (3) modified the method, applied it to cod fish, and noted an 8-fold increase in sensitivity. With some slight modification this method was adapted to crabmeat and collaboratively studied.

Collaborative Study

Eleven collaborators representing 7 Food and Drug laboratories, 2 Bureau of Commercial Fisheries laboratories, and 1 university laboratory participated in the study.

The samples were prepared in the following manner: Fresh frozen Dungeness crab was allowed to thaw in the shell at room temperature. It was sampled organoleptically from time to time, and meat at a particular stage of decomposition was removed from the shell, ground 3 times through a meat grinder with thorough mixing, and frozen for subsequent distribution to collaborators. Collaborators were requested to analyze 6 samples:

I. Early Class 1.

II. Late Class 1.

III. Borderline Classes 1 and 2.

IV. Middle Class 2.

V. Middle Class 3.

VI. Middle Class 3.

The definition of the classes conforms to the usage of Hillig et al. (4).

METHOD

(Use NH_3 - free water throughout; ordinary distilled water is suitable.)

Reagents

(a) Bromine solution.--Dissolve 1.0 ml bromine in 100 ml 0.4N NaOH (solution is stable 3 days in dark bottle).

(b) Thymol solution.--10% w/v in ethyl alcohol (solution is stable 3 days in dark bottle).

(c) Ammonia standard solution.--Dissolve 0.314 g NH_4Cl, previously dried 1 hr at 100°C, in water and dilute to 100 ml. Transfer 1.0 ml to 100 ml volumetric flask and dilute with water (10 µg NH_3/ml).

(d) n-Butyl alcohol.

(e) 3N NaOH.

(f) Phosphotungstic acid.--2.5% w/v aqueous solution.

Procedure

Place 20 g prepared sample in 500 ml conical glass-stoppered flask. Add 180 ml phosphotungstic acid solution. Shake vigorously 2 min and filter through paper (Whatman No. 1, or equivalent), collecting filtrate in 250 ml glass-stoppered conical flask. Pipet 1.0 ml filtrate (representing 0.1 g crabmeat) into 50 ml glass-stoppered conical flask. Save remainder of filtrate.[1] Add 1.0 ml phosphotungstic acid solution to separate flask for blank.

To each flask add 9.0 ml water; then add 2.0 ml thymol solution and 5.0 ml bromine solution in that order, mixing thoroughly after each addition. Let stand 10-15 min. Add 1.0 ml 3N NaOH, mix, and let stand at least 20 min.

Transfer each solution to separate 125 ml separatory funnel. Rinse flasks with 10 ml water, adding rinse to proper funnel. Add 30.0 ml n-butyl alcohol and shake vigorously. Discard lower aqueous phase and drain alcohol into glass-stoppered conical flask through glass funnel plugged with glass wool and containing ca 45 g anhydrous sodium sulfate. Measure absorbance of solution at 682 nm in 1 cm cell with blank in reference cell.

Standard Preparation

Transfer 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 ml standard ammonia solution to separate 50 ml glass-stoppered conical flasks. Add 1.0 ml phosphotungstic acid solution to each flask. Dilute contents in each flask to 10 ml with water and proceed with preparation of standard solution as in procedure, beginning with "then add 2.0 ml thymol solution...". Using portion of 0 standard as blank, measure absorbance of all 8 standard solutions at 682 nm in 1 cm cell against blank in reference cell.

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[1] If final absorbance reading is > 1.0 absorbance unit, dilute filtrate with phosphotungstic acid solution so that 1.0 ml dilute solution will produce an absorbance reading below this level.

Results and Discussion

To minimize errors resulting from reading concentrations from a graphic representation of the standard curve, we decided to derive the equation of the standard curve by the least squares method and to obtain the concentration values by substituting sample absorbances in the equation derived. To reduce calculation errors, collaborators were asked to report absorbance values only for the standards and absorbance values and dilutions performed for the samples. All calculations converting absorbance values to μg NH_3/g crabmeat were performed by the Associate Referee.

It soon became apparent that we would need to be cautious about evaluating the results and drawing conclusions. A majority of the collaborators in some way did not follow the instructions. In several cases the method was modified; in others several runs were reported so that the Associate Referee had to select the data to be used in the study.

Nevertheless, each set of results was carefully scrutinized and a series of values from each collaborator was selected that was thought to be representative of the method as it was intended to be performed.

Table 1 lists the amount of ammonia found in the crabmeat samples in μg NH_3/g crabmeat.

On the basis of the two-tailed ranking test for 11 laboratories and 6 materials (5), results from Collaborator 8 were eliminated from further calculations. The means with their standard deviations for the remaining samples are shown in Table 2.

Certain preliminary conclusions may be drawn from the data obtained in this study. There appears to be a direct relationship between the amount of ammonia present in crabmeat and its stage of decomposition, according to organoleptic criteria. However, the results do not present as sharp demarcations between classes of decompositon as might be desired. It can be seen by examining the data for the ammonia content (Table 2) that values for Early Class 1 and Late Class 1 overlap; in fact, the mean value for Late Class 1 is less than that of Early Class 1, though the standard deviation for Early Class 1 is greater, indicating a greater scatter of those values. Borderline Classes 1 and 2 also overlap in many cases with Early and Late Class 1, though the average is somewhat higher. On the basis of mean values, there seems to be a better spread between the value of ammonia in the samples in Middle Class 2 and those in Borderline Classes 1 and 2, and also between the values of Middle Class 2 and Middle Class 3.

Table 1. Collaborative results of ammonia content in 6 samples, μg NH_3/g crabmeat

Coll.	Early Class 1	Late Class 1	Borderline Classes 1-2	Middle Class 2	Middle Class 3	Middle Class 3
1	373	342	389	1311	2012	1972
2	485	300	604	513	1730	1665
3	308	286	449	756	1853	1800
4	234	234	346	1271	1185	1862
5	320	306	566	1174	1268	668
6	249	316	277	502	1197	1128
7	265	299	367	695	1877	3185
8[a]	634	380	742	2278	2521	3385
9	413	363	581	1237	2684	2819
10	259	256	347	906	1860	1692
11	370	198	334	1330	2470	2390

[a]These results eliminated from further calculations.

Table 3 lists the ranges of values obtained ± one standard deviation (68% of the population) and ± two standard deviations (95% of the population) of the mean. It can be seen for example, that selecting the value 600 μg NH_3/g crabmeat as the dividing line between definite decomposition (Class 2) and undecomposed meat (Class 1) would yield useful results in 68% of the cases, since no undecomposed samples have values higher than 543 and no decomposed samples have values lower than 636. The more realistic 95% criterion, however, shows overlap, with some undecomposed meat classed as decomposed and some decomposed classed as undecomposed. If a limiting value were set at approximately 800 μg NH_3/g crabmeat, it would not include all decomposed samples, but no good or borderline samples would be rejected.

Most collaborators found some difficulty reproducing the standard curve. The actual values of the absorbances of duplicates varied, as well as the slopes, both on repetition by the same investigator and by comparison with other investigators. Variation in replicate sample

Table 2. Ammonia content of crabmeat according to its stage of decomposition

Class		Mean, μg NH_3/g	Std Dev.
Early Class 1	(n-10)	328	82
Late Class 1	(n-10)	290	49
Borderline Classes 1-2	(n-10)	426	117
Middle Class 2	(n-10)	970	334
Middle Class 3	(n-20)	1866	621

Table 3. Range of ammonia content of crabmeat according to its stage of decomposition

Class	Mean, μg NH_3/g ±1 Std Dev.	±2 Std Dev.
Early Class 1	246 ± 410	164 ± 492
Late Class 1	241 ± 339	192 ± 388
Borderline Classes 1 and 2	309 ± 543	192 ± 660
Middle Class 2	636 ± 1304	302 ± 1638
Middle Class 3	1245 ± 2487	624 ± 3108

color formation paralleled that of the standard solutions. Such variation seems to account for the relatively large standard deviations of the ammonia content values and hence for the limitation of usefulness of the method, as it stands, in assigning the state of decomposition to the sample. This defect was not apparent to the Associate Referee in his preliminary investigation where relatively good reproducibility on repeated analyses had been obtained.

More work is needed to indicate the cause for the variation in color formation. For example, the bromine solution must be definitely alkaline for consistent color development to take place. Further modifications of conditions - reagents, concentrations, manipulations - must be studied to assure greater reproducibility of results.

Recommendations

It is recommended that further work on details of the method be undertaken, and that with the achievement of greater reproducibility and precision an additional collaborative study be initiated.

Acknowledgments

The Associate Referee wishes to thank the following collaborators: J. N. Gaffke, Jr., Seafoods Laboratory, Oregon State University, Astoria, Ore.; J. Spinelli, Bureau of Commercial Fisheries, Seattle, Wash.; M. H. Thompson, Bureau of Commercial Fisheries, Pascagoula, Miss.; and the following members of the Food and Drug Administration: T. S. Smith, Baltimore; W. A. Hallam, Boston; B. L. McGill, Dallas; R. M. Hoss, Kansas; B. G. Miles, New Orleans; A. Weber, New York; D. L. Andersen and S. E. Artoe, Seattle.

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SPECTROFLUOROMETRIC DETERMINATION OF RAUWOLFIA SERPENTINA TABLETS AND WHOLE ROOT

By Charles C. Clark, Baltimore District

Rauwolfia serpentina, the powdered root obtained from the plant of the same name, is employed as a hypotensive.

An assay has been developed in this laboratory for Rauwolfia serpentina in both ground whole root and dosage forms. It combines modern extraction techniques with the sensitivity of fluorescence.

Identical assays are provided for both the raw material and the dosage forms in the NF XII and the 10th edition of Official Methods of Analysis of the AOAC. The method is capable of yielding consistent and reliable data if large amounts of highly colored tablet excipients are not present. However, the method is time-consuming, occupying a working day in excess of 8 hr, and uses a 4 hr Soxhlet extraction, 30 shakeouts, a solvent evaporation step, and a 20 min derivative-formation time in a heated water bath. An amount of sample equivalent to 50 tablets is frequently needed for a single assay. Sample blank absorbance values frequently account for 30% or more of the total absorbances obtained.

An improvement over this official method, in terms of time required, is the method of Kunze, Barkan, and Banes (1). This method eliminates the Soxhlet extraction and the solvent evaporation steps but maintains the 30 shakeouts, heated water bath, and large amount of sample needed.

Reserpine and rescinnamine are the principal active alkaloids in Rauwolfia serpentina, with trace amounts of other alkaloids also present (2). Any unofficial assay devised for this product would have to measure these nitrite-sensitive alkaloids in the same ratio as in the official assay. The problem, then, is essentially one of determining simultaneously 2 different active ingredients present in varying proportions.

Fluorometric excitation and emission spectra of both reserpine and rescinnamine derivatives have been published (3, 4). Both alkaloids have identically shaped emission spectra with their maximum emissions at the same wavelength (Fig. 1). The excitation spectra (Fig. 2) of the 2 alkaloids are not identically shaped but do have the maximum fluorescences at the same wavelength. Work in this laboratory also demonstrated that, if the excitation wavelength is held at this maximum, the ratio

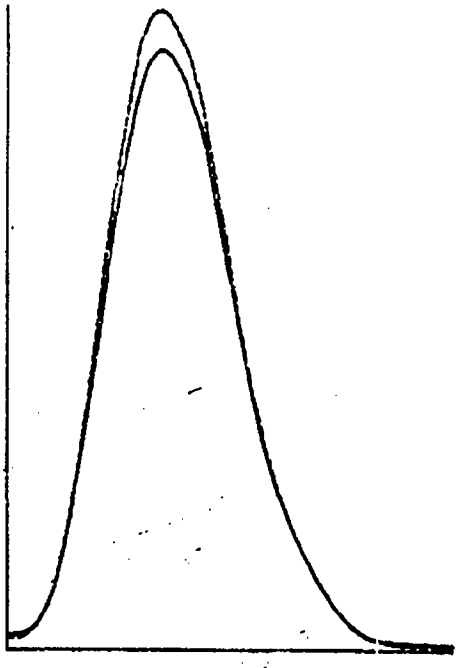


FIG. 1--Emission scan of rescinnamine (----) and reserpine (——), 420-660 nm (both compounds about 0.2 μg/ml).

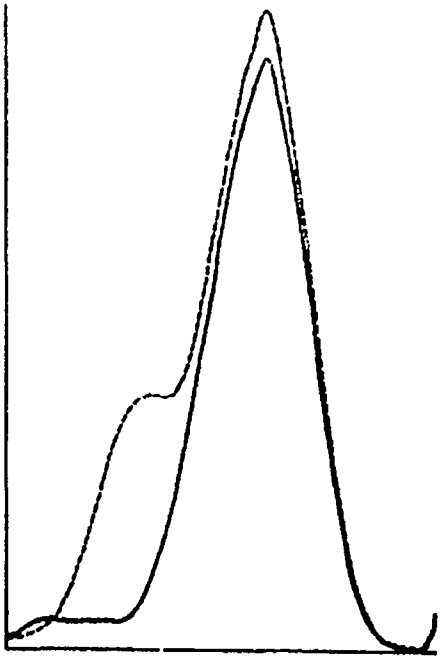


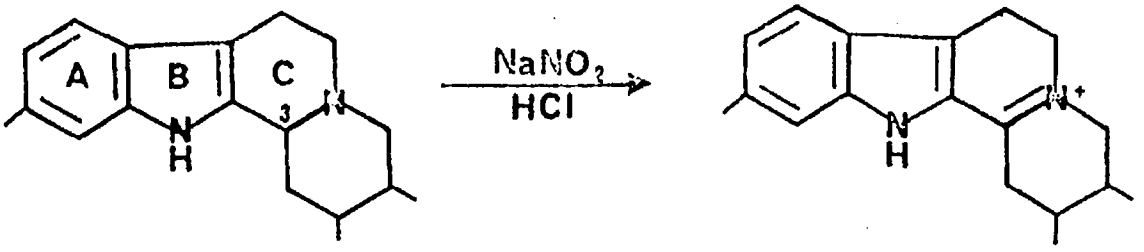
FIG. 2--Excitation scan of rescinnamine (----) and reserpine (——), 420-660 nm (both compounds about 0.2 μg/ml).

of the fluorometric responses of the nitrous acid derivatives of these 2 alkaloids are in the same ratio as their absorbances at 390 nm. This information indicated that a fluorometric method for Rauwolfia serpentina was feasible.

The nitrous acid derivative, thought to be the 3-dehydro form of the parent molecule (Fig. 3) is about 12 times as fluorescent as the parent molecule (5). The difference in spectra between the nitrous acid derivative and the parent molecule is probably due to epimerization at C_3 and unsaturation in ring C.

The strong fluorescence of this derivative allows one to use a much smaller sample and eliminates the solvent evaporation step. The smaller sample can be refluxed directly in the solvent, thus eliminating the Soxhlet extraction apparatus.

The derivative formation follows the USP XVII method for reserpine. These conditions have been established as optimum for the reserpine assay (6). Work in this laboratory indicates that these conditions are also suitable for the determination of rescinnamine even though it has a slightly different derivative formation rate as shown in Fig. 4.



Reserpine 3-Dehydroreserpine

FIG. 3--Derivative formation of reserpine.

Rauwolfia serpentina tablets are frequently coated with a dye which is not removed in the sample cleanup. The presence of dyes can lead to low results by absorption of light at the activation and/or emission wavelengths. An internal standard is incorporated in this assay to compensate for this absorption. A sample blank is also employed to correct for fluorescing products, such as oxidation products of reserpine and rescinnamine, originally present in the sample and not removed in the cleanup.

Method

Reagents

(a) Methanolic HCl.--Add 25 ml concentrated HCl to 75 ml methanol Cool to room temperature before use.

(b) Sodium nitrite solution.--Dissolve 300 mg $NaNO_2$ in 50 ml water and dilute to 100 ml with methanol. Prepare fresh daily.

(c) Sulfamic acid solution.--Dissolve 5 g sulfamic acid in 100 ml water.

(d) Standard solution.--(A) Dissolve 6.25 mg reserpine in 10 ml $CHCl_3$ and dilute to 100 ml with methanol. (B) Dilute 10 ml (A) to 100 ml with methanol. (C) Dilute 10 ml (B) to 100 ml with methanol. Solutions (B) and (C) are stable at least 1 month if kept in the dark.

Apparatus

Chromatographic column.--Lower layer: 3 g Celite plus 2 ml saturated $NaHCO_3$ solution. Upper layer: 3 g Celite plus 2 ml 0.5N H_2SO_4.

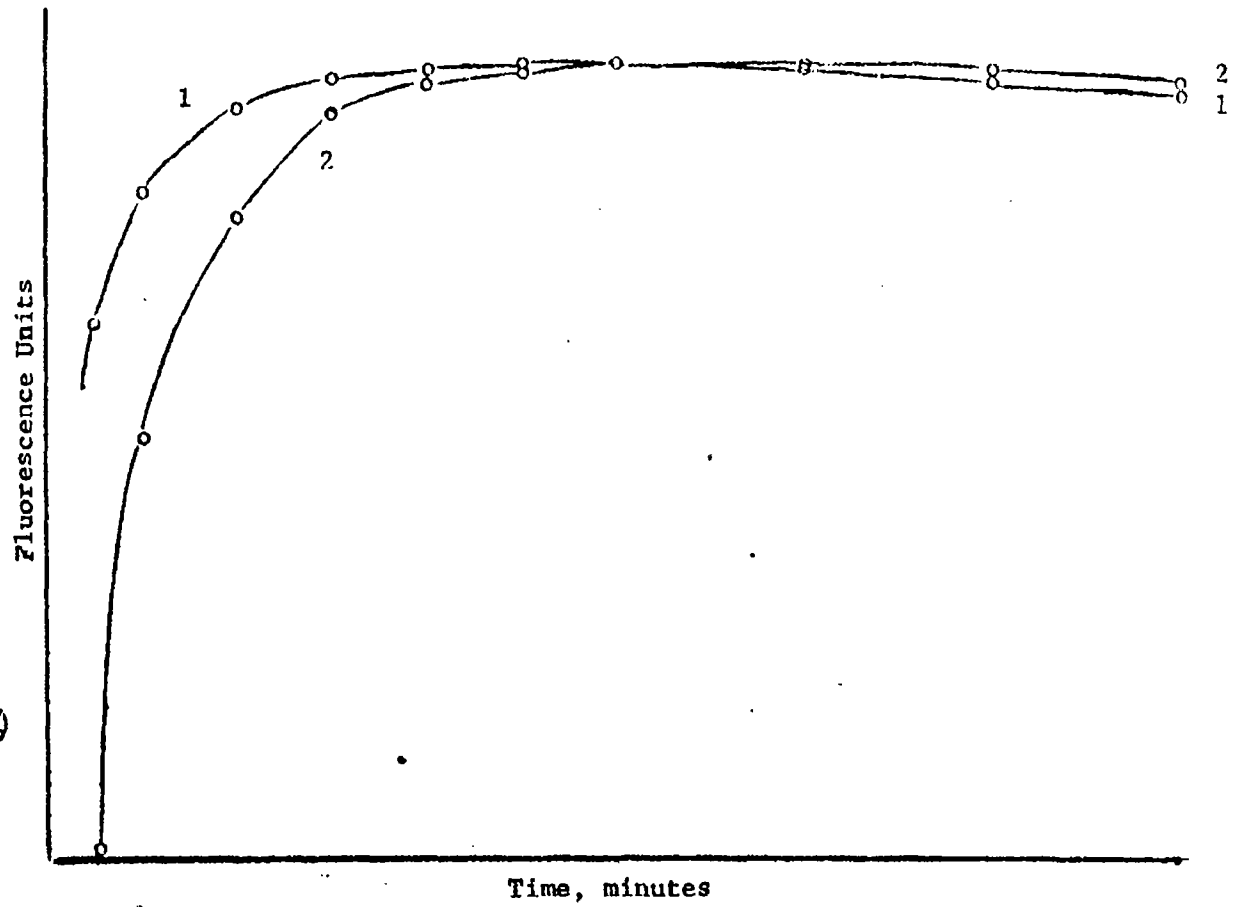


FIG. 4--Derivative formation rate for reserpine (1) and rescinnamine (2).

Procedure

Powder a minimum of 20 tablets and pass through 60 mesh sieve. Mix composite thoroughly. Weigh amount of sample equivalent to ca 250 µg total alkaloids into 250 ml Erlenmeyer flask equipped with air condenser. Add 100 ml 95% ethanol and reflux on steam bath 30 - 60 min. Cool to room temperature, add 100 ml $CHCl_3$, stopper securely, and shake solution vigorously 30 min. Transfer to 250 ml volumetric flask, dilute to volume with $CHCl_3$, and filter through paper. Transfer 50 ml aliquot of filtrate to 250 ml separatory funnel containing 200 ml 0.5N H_2SO_4 and shake funnel vigorously 2 min. Drain lower layer (may be slightly turbid) onto chromatographic column. Collect eluant in 200 ml volumetric flask containing 50 ml methanol. Extract aqueous solution in separatory funnel with 4 portions of 25 ml $CHCl_3$, draining each extract onto column after previous one enters column. Dilute eluant to volume with $CHCl_3$ and mix.

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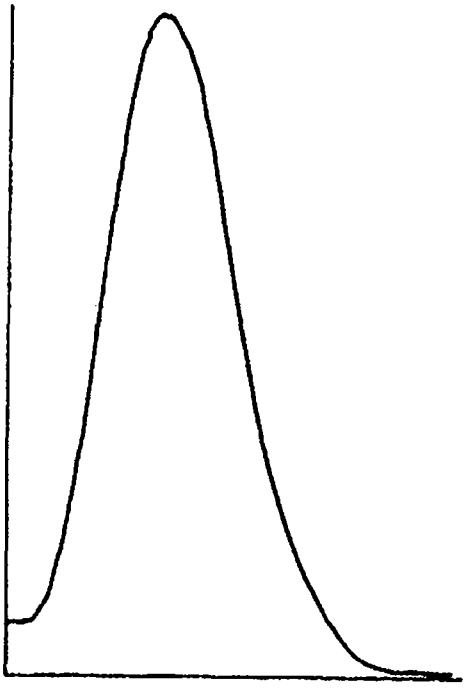

FIG. 5--Emission spectrum of a typical sample of Rauwolfia serpentina, 420-660 nm.

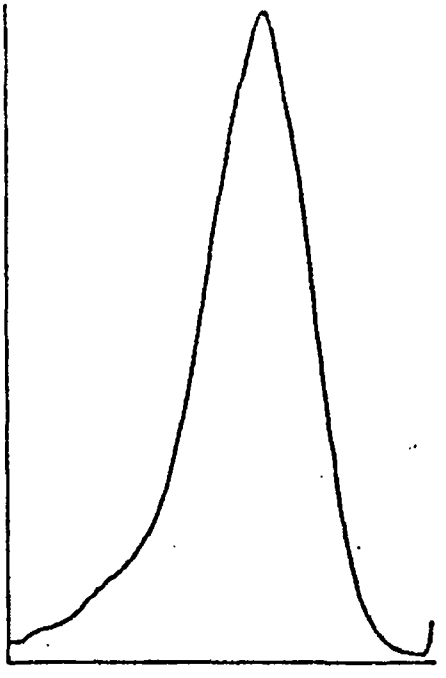

FIG. 6--Excitation spectrum of a typical sample of Rauwolfia serpentina, 260-480 nm.

Transfer 25 ml aliquots of this solution to each of three 50 ml volumetric flasks labeled A, B, and C. To flasks A and B add 10 ml methanol. To flask C add 10 ml standard solution C. Add 5 ml 50% aqueous methanol to flask A. To flasks B and C add 5 ml $NaNO_2$ solution. Add 5 ml methanolic HCl to all 3 flasks. Mix thoroughly. After 30 min, add 2.5 ml sulfamic acid solution to all 3 flasks and dilute samples to volume with methanol. Mix and determine fluorescence after 15 min, mixing and venting solution to dispel nitrogen during this time.

Use the following instrumental conditions: activation wavelength 400 nm (uncorrected), emission wavelength 502 nm (uncorrected). Use minimum activation and emission slit widths consistent with good quantitation according to manufacturer (on our Hitachi Perkin-Elmer MPF-2A, this is 10 nm and 12 nm, respectively). The sensitivity used is dependent upon slit widths.

% Reserpine - rescinnamine group alkaloids =

$$\frac{\text{Sample B - Sample Blank A}}{\text{Standard C - Sample B}} \times \frac{\text{mg Standard}}{\text{g Sample}} \times \frac{\text{(Av. g/Tablet) x 4}}{\text{mg Tablet declaration}}$$

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Results

Figures 5 and 6 show typical emission and excitation spectra of Rauwolfia serpentina samples. Results are presented in Table 1 for the analysis of tablets by both the NF XII and fluorometric methods.

Table 1. Comparison of NF XII and fluorometric method

	Rauwolfia serpentina, %	
Sample[a]	NF XII	Fluorometric Method
1	0.192	0.191 0.192 0.193 0.190
2	0.150	0.152
3	0.138	0.140
4	0.160	0.162
5	0.150	0.152
6	0.186	0.188

[a]Sample 1 is Rauwolfia serpentina NF. All other samples are red sugar-coated tablets.

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APPLICATION OF NEUTRON ACTIVATION ANALYSIS TO FOOD PRODUCTS: A BIBLIOGRAPHY

by James T. Tanner and Melvin Friedman
Division of Food Chemistry and Technology

For about 4 years the Food and Drug Administration, utilizing the Naval Research Laboratory reactor, has applied neutron activation analysis to various samples of foods, drugs, and biological materials. Neutron activation analysis is especially attractive for measuring both trace and major amounts of elements in foods: (1) It is a sensitive method of analysis; (2) it is free of reagent and laboratory contamination; and (3) the method of measurement depends on the nuclear properties of the element and consequently matrix effects are unimportant.

In the past 10 years many research workers have come to realize the importance of the application of neutron activation analysis to food products and so the literature in the field has burgeoned. This bibliography was compiled so that research workers interested in the application of neutron activation analysis to foods could have more ready access to relevant literature.

This bibliography derives from a search of Nuclear Science Abstracts through November 30, 1969 and therefore covers the literature through September 1969. The key words used were: activation analysis, animal feeds, dairy products, diet, food, food chains, fruits, meats, nutrients, and vegetables.

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Interbureau By-Lines No. 5
March 1970

POLAROGRAPHIC DETERMINATION OF NITRATES IN AQUEOUS MEDIA CONTAINING NITRITES

Albert L. Woodson, Chicago District

A drug formulated to contain the following ingredients per tablet was assayed for nitrate and nitrite content:

Sodium nitrite	65 mg
Potassium nitrate	135 mg
Powd. Ext. Echinacea	6.5 mg
Powd. Ext. Chionanthus	6.5 mg
Powd. Ext. Iris	6.5 mg
Sodium Glycocholate	6.5 mg

The nitrite was satisfactorily assayed by an AOAC colorimetric procedure (1).

For the assay of the nitrate concentration the phenoldisulfonic acid procedure (2) was modified to eliminate the interference of nitrite ions. This modification consisted of treating the acidified solution of the sample with urea, which destroys the nitrite ion by converting it to nitrogen. The nitrate assay value obtained by this modified procedure was low: 75% of the label declaration. For a check analysis, the author successfully utilized aqueous polarography for the nitrate assay.

This polarographic assay is based on the catalytic effect of the nitrate concentration on the polarographic reduction wave of uranium (3).

A 2×10^{-4} M solution of uranyl acetate, $UO_2(C_2H_3O_2)_2$, in 0.01M HCl-0.1M KCl yields two polarographic reduction waves at approximate half-wave potentials -0.2 and -0.9 v vs. the standard calomel electrode. The wave at -0.2 v represents the reduction of uranium (+6) to uranium (+5); the wave at -0.9 v represents the reduction of uranium (+5) to uranium (+3) (3). In the presence of nitrate ions the uranium wave at -0.9 increases (3); as the uranium ions are reduced, these reduced ions are immediately reoxidized by the nitrate ions. These reoxidized uranium ions are reduced again and this pattern of polarographic reduction-nitrate oxidation-polarographic reduction is continued until the solution has been depleted of the oxidizer, the nitrate ions. The net result is an increase in the height of the second wave in the polarographic reduction profile of uranium. This catalytic effect of the nitrate ion is proportional to the concentration of nitrate ions as low as 5×10^{-4} M (3).

DSW 033468

The nitrite ion in aqueous acid media forms nitrous acid, which is polarographically reducible at -1.0 v. Therefore, any polarographic assay of a mixture of nitrate and nitrite ions would be inaccurate because of the close proximity of the two half-wave potentials: -0.9 v for the nitrate ion and -1.0 v for the nitrite ion. However, since nitrous acid is easily destroyed by sulfamic acid (it converts nitrous acid to nitrogen), the addition of sulfamic acid to the solution before polarography eliminates the interference of the nitrite ion (3).

Method

Reagents and Apparatus

(a) Sulfamic acid solution.--About 0.75M.

(b) Uranyl acetate.--$UO_2(C_2H_3O_2)_2.2H_2O$, Fisher Certified Reagent.

(c) Potassium nitrate.--Fisher Primary Standard.

(d) Sargent XV polarograph.--With standard calomel reference electrode.

Solutions

(a) Polarographic electrolyte.--Dissolve 86.9 mg uranyl acetate in 1 L solution containing 0.01M HCl and 0.1M KCl. The uranyl acetate concentration of this solution is 2×10^{-4}M.

(b) Standard potassium nitrate.--Dissolve 97.8 mg primary standard in 100 ml water. This solution contains 0.978 mg KNO_3/ml.

Procedure

Accurately weigh portion of powdered sample equivalent to ca 130 mg potassium nitrate into 100 ml volumetric flask. Add ca 30 ml water and shake on mechanical shaker 15 min. Dilute with water to volume, mix well, and filter.

Add 2 ml aliquot of filtered sample solution, 1 ml water, 2 ml sulfamic acid solution, and 15 ml polarographic electrolyte to small Erlenmeyer flask. Mix well, let stand 10 min, de-aerate with nitrogen 5 min, and then polarograph. Scrub nitrogen before introduction into polarographic cell by passing it through scrubber containing polarographic electrolyte.

Determine recovery as follows: Mix 2 ml filtered sample solution, 1 ml standard potassium nitrate solution, 2 ml sulfamic acid solution, and 15 ml polarographic electrolyte. Run sample and recovery determinations in triplicate.

Plot height of diffusion current vs. mg potassium nitrate/ml. Measure diffusion current at -1.1 v as indicated in Fig. 1.

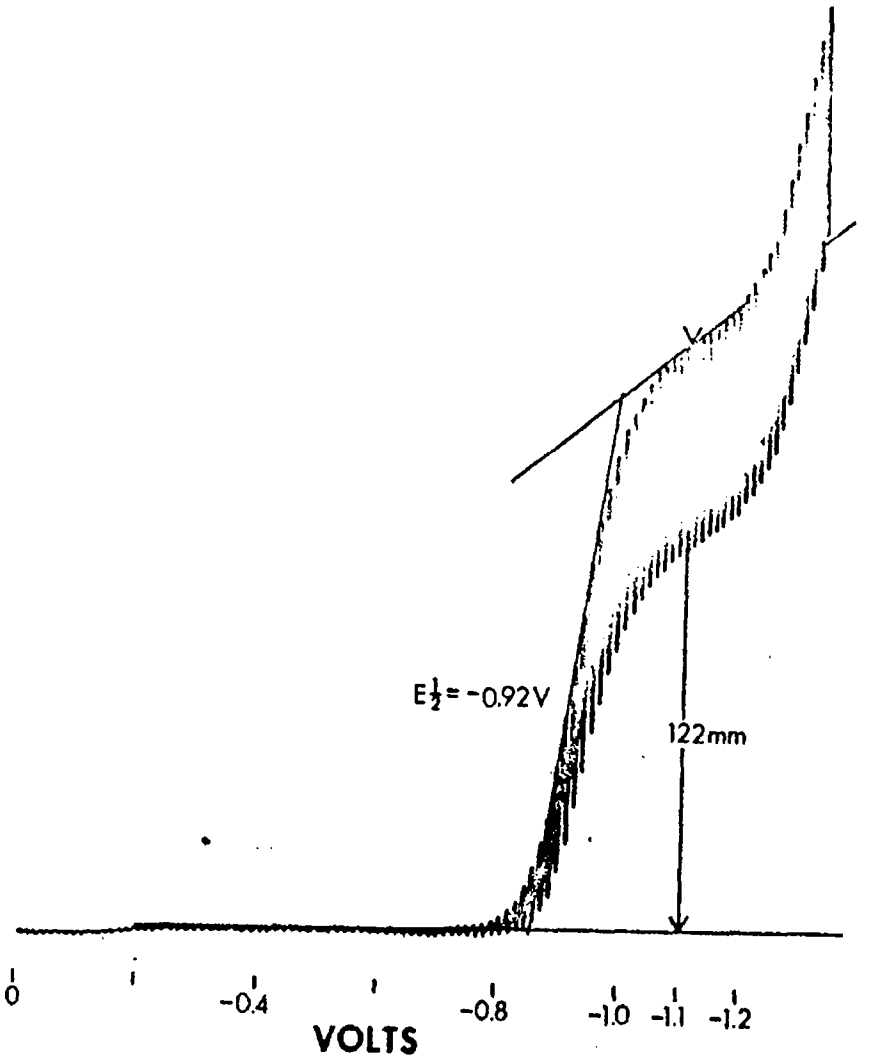

FIG. 1--Second wave in the polarographic reduction profile of uranium.

Results and Discussion

The results of the sample assays are shown in Table 1; the results of the recovery determinations are shown in Table 2. These data indicate that this procedure may be successfully employed for the polarographic determination of nitrates in the presence of nitrites.

The preparation of a standard potassium nitrate curve shows the linearity of the nitrate concentration-wave height profile in the range of 0.078-0.195 mg/ml (Fig. 2).

Table 1. Results of polarographic assay of sample

Sample	E 1/2, volt	mg/Tablet		% of Declaration
		Found	Declared	
1	-0.90	129.1	130	99.3
2	-0.90	132.0	130	101.5
3	-0.90	130.3	130	100.2
Av.	-0.90	130.5	130	100.3

A typical polarographic wave obtained with the sample solution is shown in Fig. 1. The wave at -0.2 v, which represents the reduction of uranium (+6) to uranium (+5), is not seen on this typical polarogram because the sensitivity of the Sargent XV has been lowered in order to confine the wave at -0.9 v to the available chart space. If the sensitivity of the instrument were set at 0.04 μA/mm, the wave at -0.2 v would appear, but the wave at -0.9 v would be off the chart.

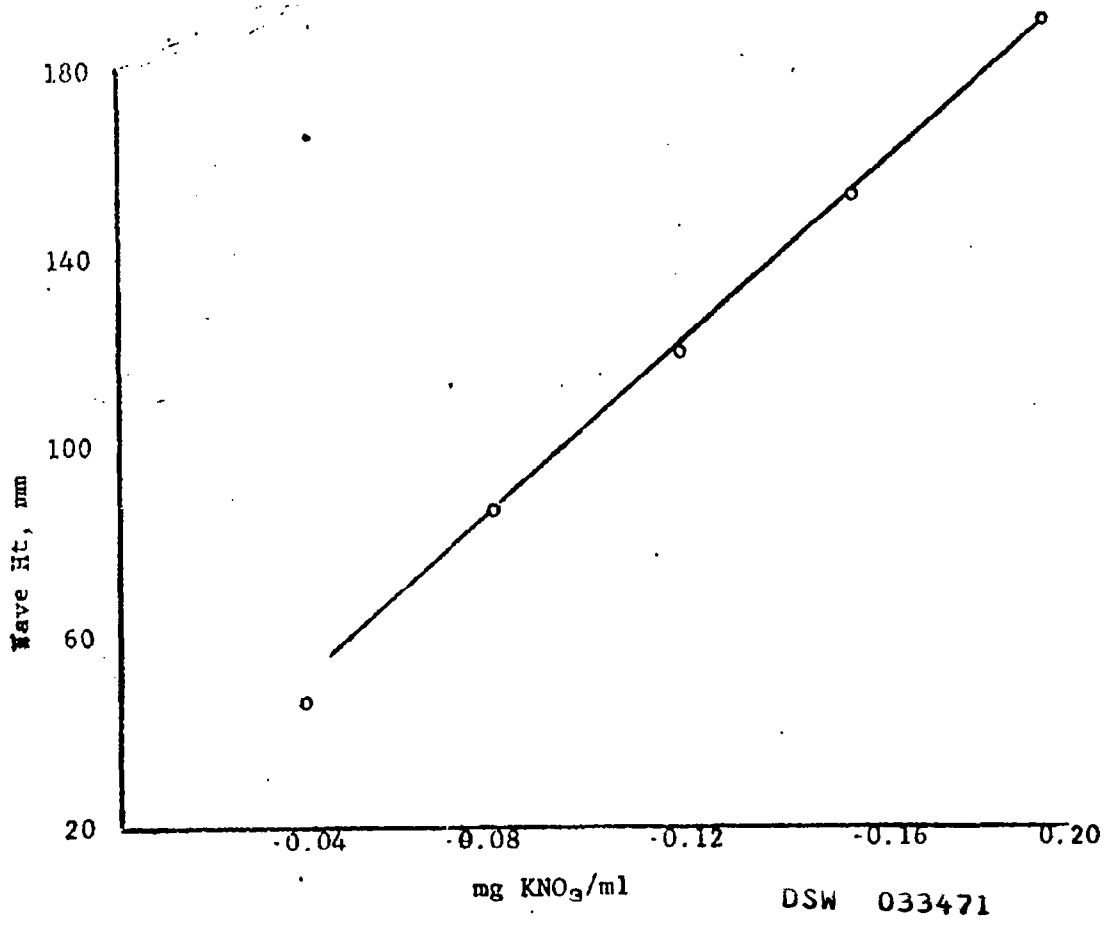

FIG. 2--Nitrate concentration vs. wave height profile.

Table 2. Results of recovery of nitrate by polarographic assay

Assay	E 1/2, volt	mg KNO_3 Added	mg KNO_3 Found	% Recovery
1	-0.91	0.978	0.96	98.2
2	-0.92	0.978	0.98	100.2
3	-0.91	0.978	0.96	98.2
Av.	-0.913	0.978	0.967	98.9

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Interbureau By-Lines No. 6
May 1970

CORRELATION OF ORGANOLEPTIC EVIDENCE WITH pH, VOLATILE BASES, AND INDOLE AS INDICES OF DECOMPOSITION IN RAW, FROZEN SHRIMP

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Food scientists are in need of a unified scientific approach to determining the state of decomposition of shrimp. No single approach - organoleptic, bacteriological, or chemical - has been devised which is completely satisfactory for such a purpose.

This study was undertaken to determine if any direct correlation exists between organoleptic classification and values for pH, volatile base, and indole as obtained from a single portion of shrimp. Such examinations have previously been conducted on shrimp, but to our knowledge not on the same portion of shrimp; therefore, no direct correlation of values can be made (1).

Previous investigators have reported two principal types of decomposition in raw shrimp: putrefactive and ammoniacal. The putrefactive type occurs when shrimp, before icing, are exposed to a temperature favorable for bacterial incubation. Once started, this type of decomposition proceeds fairly rapidly even though the shrimp are subsequently well iced. Ammoniacal decomposition occurs in shrimp which have been held under well iced conditions until decomposed. This type of decomposition is slow and is characterized by an odor of free ammonia, in addition to other odors associated with decomposition (2).

The shrimp used in this study were of the putrefactive type and were organoleptically classified as follows:

Class I - No odor or a slight fish odor characteristic of the usual commercial product.

Class II - Decomposed. A slight but definite odor recognizable as indicative of decomposition.

Class III - Advanced decomposition. An intense persistent odor recognizable as indicative of pronounced decomposition, definite and unmistakable.

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[1]Present address: San Juan Section, New York District. This paper was submitted as a progress report of the Associate Referee on Decomposition in Shellfish.

These shrimp (white, brown, and pink) were commercially available raw, frozen, peeled, and deveined. They were hard frozen when received.

Shrimp were thawed and individually classified organoleptically. Individual 50 g portions of Class I, II, or III were weighed and used to determine pH, volatile base, and indole. When determinations could not be made on the same day as the organoleptic examination, individual 50 g portions were immediately hard frozen and stored; when time permitted, they were thawed, classified organoleptically, and used to determine pH, volatile base, and indole. In no case did more than a week elapse between the original organoleptic examination and other tests.

Since published procedures were not available for determining a series of indices on the same 50 g portion, established methods for indole (2) and volatile bases (3) were combined and modified as follows:

(A) Distillation procedure: (1) A calcium hydroxide trap was placed between distillation flask and condenser. (2) Antifoam A spray was used instead of alcohol in the distillation flask.

(B) Volatile bases (3): The distillate was collected in 0.1N HCl and back-titrated with 0.1N NaOH to pH 7, using a pH meter with a glass and calomel electrode.

(C) Indole (4): (1) The titrated volatile bases distillate was quantitatively transferred to a 1000 ml separatory funnel containing 10 ml of 10% HCl and 10 ml of a saturated solution of sodium sulfate. Three 1-minute extractions were made with 30 ml portions of $CHCl_3$. The $CHCl_3$ extracts were drained into a 125 ml separatory funnel through an absorbent cotton plug. (2) Color was developed as in the established indole method.

Experimental

Reagents and Apparatus

(a) Color reagent, purified acetic acid, dilute hydrochloric acid, and indole standard solution.--All from sec. 18.036 (4).

(b) Distillation apparatus.--(1) A separate steam generator should be used for each unit. Steam generator may be made from 2 L Erlenmeyer flask connected to all-glass steam distillation apparatus, ammonia distilling apparatus (JD-1710 Scientific Glass Apparatus Co., Inc., Bloomfield, N. J., or equivalent with minimum use of rubber tubing). (2) Distilling flask. (3) Trap flask, 800 ml $\overline{S}$ Kjeldahl, connected through a (4) spray trap via rubber tubing to a trap flask which is connected to a (5) straight bore condenser through the spray trap. An 800 ml beaker is an effective receiver (Fig. 1). The $\overline{S}$ joint is cut away from the spray trap on the sample flask and a rubber tube is used to connect the sample spray trap to the spray trap on the trap flask.

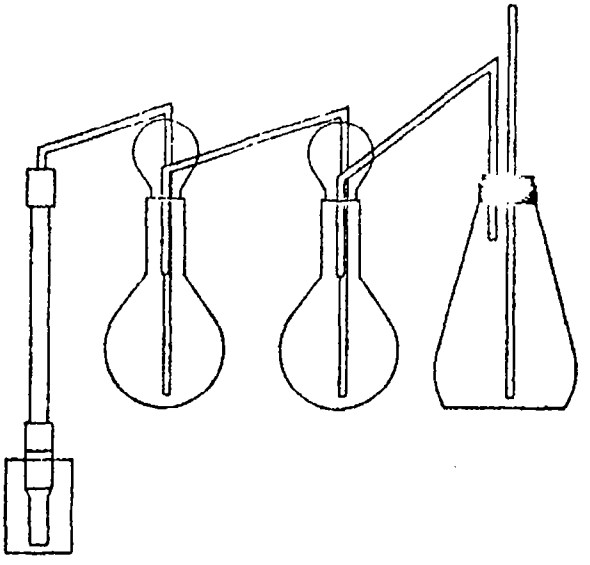

FIG. 1--Schematic of distillation apparatus.

(c) pH meter.---Reading to two decimal places (glass and calomel electrode).

(d) Calcium hydroxide.--(Baker analyzed reagent 1372 or equivalent). 15 g of $Ca(OH)_2$ is dissolved in 1000 ml of water. 150 ml is used in the trap flask for each determination.

Organoleptic Examination

The shrimp were thawed, the organoleptic examination was performed to note classification, and samples were weighed in individual 50 g portions.

pH Determination

The 50 g portions of shrimp were placed in a 1 qt. Waring Blendor and blended for 2-3 min. The pH of the blended shrimp was taken with a pH meter (glass and calomel electrode) and recorded to two decimal places.

Determination of Volatile Bases

Eighty to 100 ml of water and a 10 second burst of Dow Corning Antifoam A pressurized spray was added to the previously blended 50 g portion of shrimp and blended for 2 min. The blend was quantitatively transferred to a distillation apparatus (Fig. 1). A 500 ml portion was distilled into a beaker containing 50 ml of 0.1N HCl (condenser tip is immersed into the 0.1N HCl in the receiving beaker). (Note: The calcium hydroxide solution in the trap flask

must be boiling before distillation begins.) The distillate was titrated to pH 7 with 0.1N NaOH (pH meter), corrected for back titration, and the value multiplied by 20 to correct to the quantity of 0.01N NaOH in 100 g of shrimp ("volatile bases").

Indole Determination

The titrated distillate from the volatile base determination was transferred to a 1000 ml separatory funnel containing 10 ml of a saturated solution of sodium sulfate and 10 ml of 10% HCl. The aqueous phase was extracted 1 min with three 30 ml portions of $CHCl_3$ (USP). The $CHCl_3$ was drained through an absorbent cotton plug in the stem of the 1000 ml separatory funnel into a 125 ml separatory funnel containing 10 ml of the color reagent and shaken for 2 min. Then 9 ml of color reagent (bottom layer) was drained into a 50 ml glass-stoppered cylinder and diluted to 50 ml with acetic acid. The color developed immediately upon shaking. Using a visible recording spectrophotometer, the spectrum was recorded from 650 to 425 nm against a reagent blank. The quantitative absorbance maximum was read at 567 nm and a background correction was made by drawing a straight line from the absorbance peak at 650 nm to that at 425 nm. Background absorbance (the point at which the line crosses 567 nm) is subtracted from total absorbance at 567 nm. Indole standard solutions containing 0, 20, 30, and 40 µg indole were prepared and taken through the procedure, and an indole standard curve was prepared. The amount of indole in the shrimp sample was calculated from the standard indole curve and multiplied by a factor of 2 to obtain µg indole in 100 g of shrimp.

Recovery of indole by this method is 96.9 - 100% if the calcium hydroxide trap is boiling vigorously before distillation is begun. If the calcium hydroxide in the trap is not boiling vigorously, indole recovery values drop to 85 - 90%.

Results and Discussion

The results of the determination of the three classes of organoleptic shrimp are listed in Table 1. pH, volatile bases, and indole were determined on 35 portions of white, brown, or pink shrimp in Class I, 15 portions in Class II, and 23 portions in Class III.

The pH, indole content, and volatile bases values show a steady increase as the shrimp progress from Class I through Class III.

Table 1 also shows that the indices overlap into several organoleptic classes. Some indices do not properly represent the organoleptic classification. The reasons for this deviation are not known.

Table 1. Experimental data on white, brown, and pink shrimp (not listed separately)

pH	Indole, µg/100 g	Volatile Bases, ml 0.01N NaOH/100 g	pH	Indole, µg/100 g	Volatile Bases, ml 0.01N NaOH/100 g
Organoleptic Class I			Organoleptic Class II		
7.13	8.4	173	7.59	46.2	268
7.08	9.6	163	7.56	71.2	276
7.12	0	217	7.57	40.0	255
7.80	10	143	7.42	44.0	274
7.87	20	208	7.82	6.0	363
7.07	8	260	7.50	64	258
7.12	8.4	264	7.72	24	87
7.09	0	216	7.81	15	212
7.10	8.4	163	7.72	30.4	270
7.09	8.8	167	7.86	24	326
7.08	8.0	192	7.81	35	234
7.13	0	174	7.45	28.4	222
7.40	12.4	175	7.52	17	239
7.20	8.0	125	7.58	30.6	224
7.25	8.0	142	7.46	29.2	209
7.30	0	211			
7.48	14.0	205	Organoleptic Class III		
7.30	3.0	192			
7.52	0	33	7.67	90.0	368
7.43	0	61	7.91	156.0	366
7.35	0	68	7.99	70.0	251
7.48	0	52	7.81	400.0	422
7.30	0	37	7.98	14.0	165
7.46	0	52	7.25	54.0	298
7.70	8	150	7.90	62.0	277
7.57	4	87	7.87	25.0	144
7.26	0	119	7.45	46.0	277
8.03	11	310	7.98	19.0	206
7.31	0	297	8.22	155.8	149
7.37	0	101	8.00	171.2	326
7.47	0	166	8.02	42.0	252
7.53	0	162	7.95	26.0	230
7.62	11.4	203	7.70	128.6	359
7.43	14.0	72	7.72	120.4	399
7.40	10.4	65	7.69	102.4	340
			7.41	28.0	468
			7.70	90	503
			7.72	54.4	423
			7.60	74.0	660
			7.83	115.0	662
			7.76	94.0	715

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Table 2. Organoleptic and chemical indices of decomposition in shrimp (from Table 1)

	Class I	Class II	Class III
pH Av.	7.37	7.63	7.79
Range	7.07 - 8.03	7.42 - 7.86	7.25 - 8.22
Indole, g/100 g			
Av.	5.5	33.6	110.4
Range	0 - 20	6.0 - 71.2	14.0 - 400
Volatile Bases, ml 0.01N NaOH			
Av.	155	248	359
Range	33 - 310	87 - 363	144 - 715

Table 2 summarizes the results for pH, indole content, and volatile bases obtained on the 3 organoleptic classes of the shrimp. Separate lots of brown shrimp and pink shrimp were examined by the organoleptic technique previously discussed. The separated shrimp samples were examined immediately and then again after the same lot of shrimp was allowed to decompose in plastic bags at room temperature for 24 hr. The values for organoleptic class, pH, indole content, and volatile bases on the separated brown and pink shrimp increased as shown in Tables 3 and 4. Since this study was conducted on commercially prepared shrimp, all previous history of the shrimp is unknown. The deviations of some values could be the result of chemicals used on the shrimp, variations with type of shrimp, and general handling before and after freezing.

Conclusion

Each set of values reveals a noticeable upward trend as the shrimp decompose. These results are of value as additional data to help correlate the findings from organoleptic determination of shrimp quality. The 4 indices should be further evaluated on individual species of commercial shrimp under known conditions and beginning with live shrimp.

DSW 033478

Table 3. Organoleptic class, pH, indole content, and volatile base values for brown shrimp and the same lot held 24 hr at room temperature

pH	Organoleptic Class	Indole, ug/100 g	Volatile Bases, ml 0.01N NaOH/100 g
		Fresh Shrimp	
7.40	I	12.4	175
7.20	I	8.0	125
7.25	I	8.0	142
7.30	I	0.0	211
7.48	I	14.0	205
7.30	I	3.0	192
Av. 7.32		7.56	175
		Stored Shrimp	
7.41	III	28.0	468
7.70	III	90.0	503
7.72	III	54.4	423
7.76	III	74.0	660
7.83	III	115.0	662
7.76	III	94.0	715
Av. 7.70		75.9	572

Table 4. Organoleptic class, pH, indole content, and volatile base values for pink shrimp and the same lot held 24 hr at room temperature

pH	Organoleptic Class	Indole, µg/100 g	Volatile Bases, ml 0.01N NaOH/100 g
		Fresh Shrimp	
7.52	I	0	33
7.43	I	0	61
7.35	I	0	68
7.48	I	0	52
7.30	I	0	37
7.46	I	0	52
Av. 7.42		0	50
		Stored Shrimp	
7.57	III	12	131
7.54	III	16	159
7.60	III	14	139
7.56	III	26	180
7.62	III	20	208
7.70	III	26	155
Av. 7.60		19	162

DSW 033480

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DSW 033481

Interbureau By-Lines No. 6
May 1970

DIFFERENTIAL EFFECTS OF DDT AND POLYCHLORINATED BIPHENYLS ON THE CENTRAL NERVOUS SYSTEM

By Thomas J. Sobotka, Division of Pesticide Chemistry and Toxicology

Refined chemical procedures have only recently been developed which enable DDT to be differentiated from polychlorinated biphenyls (PCB) in single samples (personal communication from Judith Armour, DPCT, to be published in JAOAC, July 1970). Because these two classes of compounds are difficult to separate and because they are both found in wildlife species, it is possible that effects previously attributed to DDT may in fact be due to Aroclor residues. The present work was undertaken to determine whether central behavioral and physiological systems are similarly or differentially affected by DDT and polychlorinated biphenyls. Two representative samples of the PCB class were investigated, namely, Aroclor 1260 and Aroclor 1254.

Methods

Adult male mice (Flander's Research Farm) were used throughout the study. Animals were housed in groups of 15-20 with constant lighting and temperature. Food and water were provided ad libitum.

All compounds were dissolved in corn oil and administered orally at volumes equivalent to 0.01 ml/g body weight.

Open-Field Activity (Exploratory Activity). - The apparatus to measure exploration of the DDT and Aroclor 1260 groups was different from that used to gauge exploratory activity of the Aroclor 1254 groups. Therefore, only the DDT and Aroclor 1260 groups can be validly compared. However, the data from the Aroclor 1254 groups will be presented for completeness.

Exploratory behavior for the DDT and Aroclor 1260 groups was measured by the use of a large rectangular box (10 x 18 x 6") with marked grid floor. Twenty-four hours after dosing, each animal was tested individually. The number of squares traversed per minute was recorded for a 5 min period. The average minute activity was used as the exploratory activity.

Exploration by the Aroclor 1254 groups was determined in circular photoactivity chambers (18" diameter x 15" high). Twenty-four hours after dosing, each mouse was placed in a photoactivity chamber and the number of light beams crossed per minute for 5 min was recorded. The average minute activity was used as the exploratory activity.

In order to gauge the rate at which the mice were habituating to the novel environment during their 5 min exposure, the difference between the average activity of the first 2 min and the last 2 min was calculated. A large difference (the average activity of the last 2 min being smaller) was taken to imply a rapid suppression of motor exploration, i.e., a high degree of habituation; conversely, a smaller difference would indicate relatively little motor suppression or habituation to the novel environment. The use of a decline in exploratory activity over a period of time as an index of habituation is based on the methods of Carlton (1).

DDT was given in doses of 0, 1.0, 10.0, and 25.0 mg/kg (total number of mice = 60). Aroclor 1260 was administered at 0, 1.0, and 10.0 mg/kg (total number of mice = 39). Aroclor 1254 was given at doses of 0, 0.5, 1.0, and 10.0 mg/kg (total number of mice = 48).

Passive Avoidance. - Fear-induced suppression of motor activity (passive avoidance) was measured in a standard passive avoidance situation (2). The apparatus consisted of a small Plexiglas chamber (3.5 x 6.5" with a grid floor) adjoining a larger chamber (13.5 x 13.5" with a grid floor) with a door between them. Four hours after dosing, each mouse was placed in the small chamber facing away from the door. The door was immediately opened, thereby activating a timer. The time (latency) to enter the larger chamber was recorded. As soon as the animal stepped into this chamber, the door was closed and an 8 ma/0.2 sec shock was delivered through the grid floor. The mouse was then returned to its home cage. On the following day the animal was given a single post-shock (fear) test. The latency to enter the large "shock" chamber was recorded but no shock was given.

DDT (80 mice), Aroclor 1260 (81 mice), and Aroclor 1254 (48 mice) were administered in oil in single oral doses of 0, 0.5, 1.0, and 10.0 mg/kg.

Maximum Electroshock Seizure. - Four hours after dosing, maximum electroshock seizure was induced by a 30 ma/0.2 sec pulse delivered via saline wetted corneal electrodes. The durations of the following seizure components were measured: tonic flexion, tonic extension, and clonus. In addition, the ratio of tonic flexion/tonic extension was calculated.

The following doses of DDT (61 mice), Aroclor 1260 (46 mice), and Aroclor 1254 (40 mice) were given: 0, 0.5, 1.0, and 10.0 mg/kg.

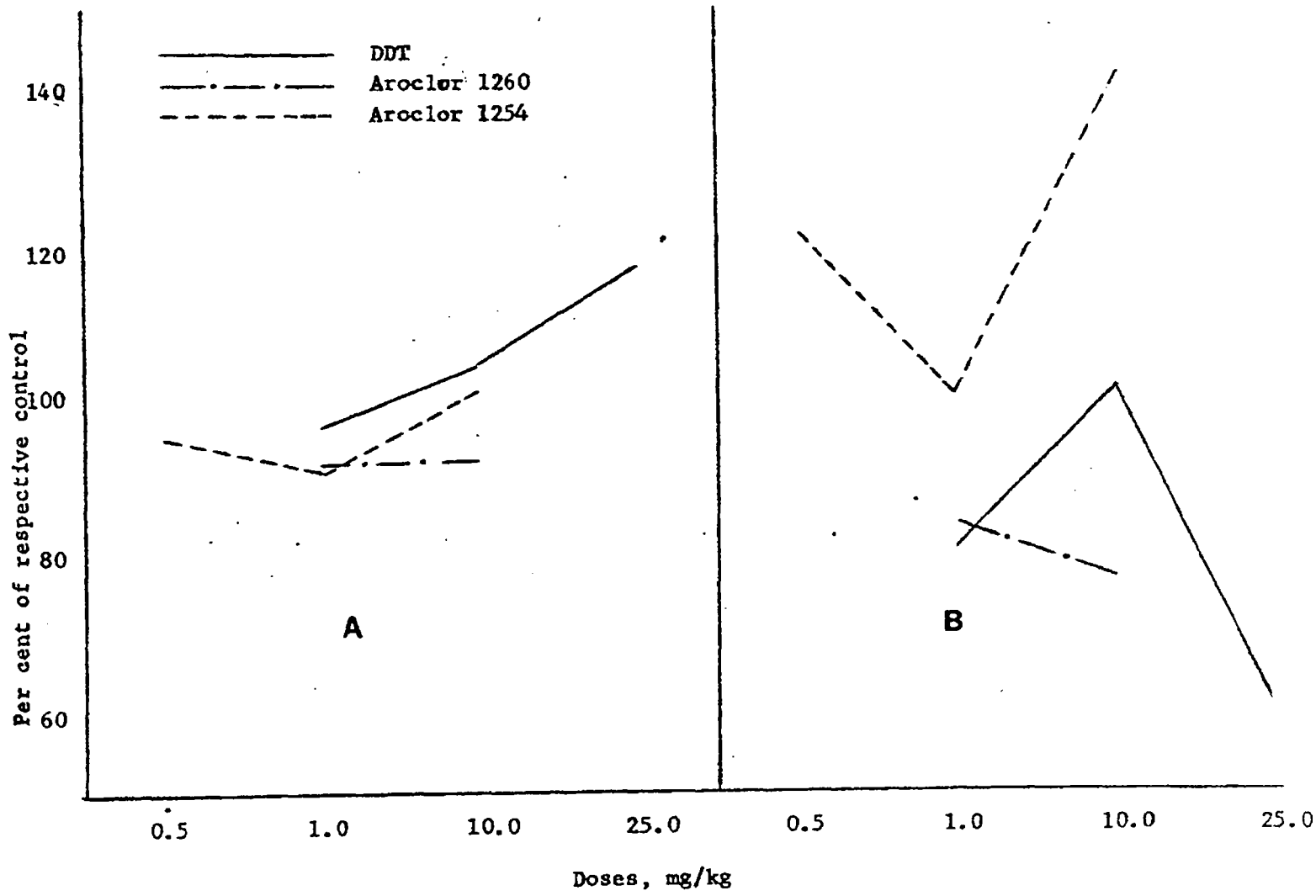

FIG. 1--Effect of DDT, Aroclor 1260, and Aroclor 1254 on (A) exploratory behavior and (B) habituation (4 hr pretreatment).

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Results

All results are expressed as per cent of control. Figure 1A depicts the changes in exploratory activity induced by DDT and Aroclor 1260. At 1.0 and 10.0 mg/kg there does not appear to be any marked differences between the effects of the 2 compounds. The Aroclor 1260 groups do, however, tend to exhibit a slightly lower exploratory activity. A similar low exploration is seen with the Aroclor 1254 group at 1.0 mg/kg.

The habituation scores seen in Fig. 1B similarly do not differentiate between DDT and Aroclor 1260. Aroclor 1254 does induce a facilitation of habituation. But this effect cannot be validly compared with the DDT or Aroclor 1260 groups since different apparatus was used.

In contrast to exploratory and habituation scores, a clear distinction between DDT and both of the Aroclor compounds is seen in the passive avoidance behavior. As shown in Fig. 2, the DDT-treated mice entered the shock (stress) chamber faster, while the Aroclor

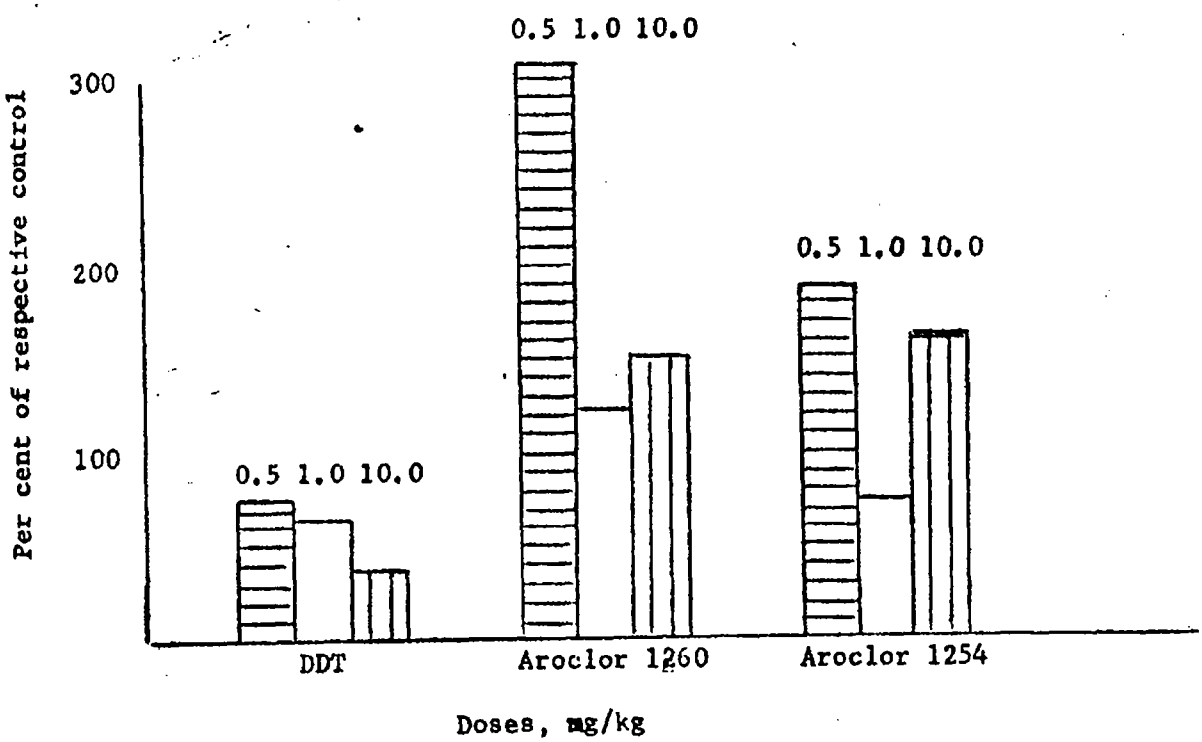

FIG. 2--Effecto of DDT, Aroclor 1260, and Aroclor 1254 on latency to enter a shock-stress chamber (all agents were given 24 hr before shock and 48 hr before testing latency to enter shock chamber).

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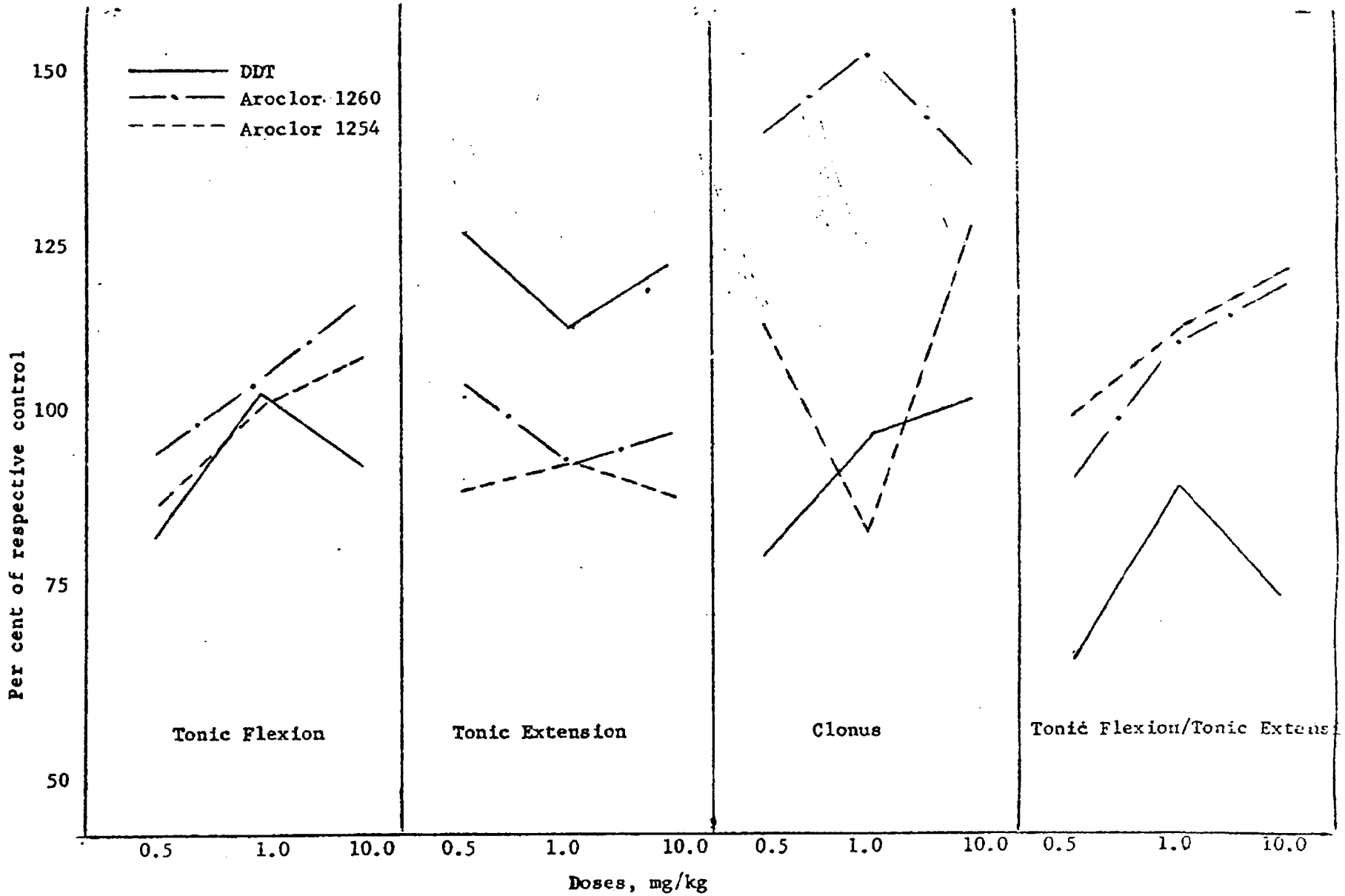

FIG. 3--Effect of DDT, Aroclor 1260, and Aroclor 1254 on maximum electroshock seizure 4 hr after dosing.

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1260- and 1254-treated animals generally took a much longer time to enter the stress chamber when compared to their respective controls.

Further distinction between DDT and Aroclor compounds can be seen in their effects on MES pattern (Fig. 3). Mice treated with either Aroclor 1260 or 1254 exhibited a prolonged duration of clonus (Aroclor 1260 was more effective in this than was Aroclor 1254) and an increased tonic flexion/tonic extension ratio, and tended to have a decreased tonic extensor duration. Each of these effects is the opposite of that found after DDT treatment. Animals treated with DDT had a prolonged tonic extensor phase and a depressed tonic flexion/tonic extension ratio, and tended to have a decreased or unchanged clonic phase compared to controls.

Discussion

The present work has shown that DDT and polychlorinated biphenyls (specifically, Aroclor 1260 and Aroclor 1254) exert differential effects on the central nervous system. The behavioral inhibition induced by shock stress (3) is attenuated by DDT as seen by the facilitation of entrance into the shock (stress) chamber. The Aroclor compounds resulted in the opposite effect. These agents prolonged the latency to enter the shock chamber, thereby enhancing the inhibitory effects of stress.

The general level of central neuronal excitability is also differentially affected. The depression of the tonic flexion/tonic extension ratio (resulting from a decreased duration of tonic flexion and a prolongation of tonic extension) by DDT reflects an overall increase in brain excitability (4).

DDT also tended to exert a depressant effect on the clonic phase of MES. In contrast, the Aroclor compounds increased the tonic flexion/tonic extensor ratio (resulting primarily from the decreased duration to tonic extension). This reflects an overall decrease in central physiological excitability. The clonic phase, which is thought to be due to an active inhibitory system (5), is generally prolonged by the Aroclor compounds, especially Aroclor 1260.

The possibility exists that DDT may attenuate central inhibitory systems, while polychlorinated biphenyls may enhance central inhibitory systems, but further experimentation is necessary.

In summary, the present work not only has shown that the differential effects exerted by DDT and polychlorinated biphenyls distinguish these two classes of compounds, but also indicates that they exert opposite effects on the central nervous system.

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[1]The index for Volume 6 is included in the cumulative index; no separate index will be printed.