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SEPARATION OF ALKYL GALLATES AS COMPLEXES WITH CINCHONINE AND STRYCHNINE

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SUMMARY

The molecular complexes of various alkyl gallates with cinchonine and strychnine are reported. It was possible to separate and characterize a gallate up to 2 μg as its complex from a mixture.

INTRODUCTION

Alkyl gallates are widely employed for extending the shelf life of various fatty food stuffs and in combination with butylated hydroxy anisole work as excellent synergists. Against certain bacteria *viz.* *Salmonera narasino*, *Saccharomyces cerevisiae* etc. they show microbicide action which increases with the increasing chain length of the alkyl radical¹. They are used as polymer stabilizers² and possess prophylactic and therapeutic antiradiation properties³. The latter effect is due to the binding of free radicals in the cells and inhibition of the activity of oxidizing enzymes⁴.

The molecular complexes of gallic acid with caffeine, theophylline and theobromine have been reported by thermal analysis⁵. Nicotine gallate is known to be insecticidal in nature⁶.

The separation and identification of alkyl gallates have been reported by employing different chromatographic techniques. Gas chromatography was carried out by using the columns packed with 1.5% SE-30, 0.7% QF-1 and 1.0% neopentyl glycol succinate polyester⁷. Reversed-phase partition chromatography⁸, paper chromatography⁹⁻¹⁸, centrifugal chromatography¹⁹ on Whatman No. 3 paper, and thin-layer chromatography using 10% acetylated cellulose²⁰, alumina²¹, silica gel²²⁻³⁵ and polyamide powder³⁶⁻³⁸ as adsorbents have been employed both for their qualitative and quantitative studies.

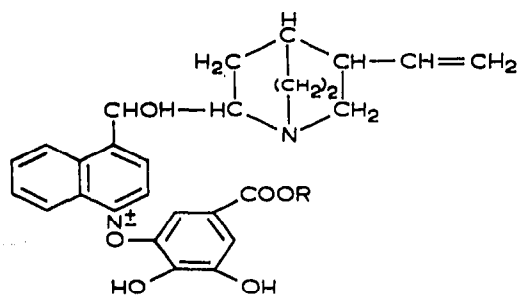
Great difficulty was faced by the present authors in the distinct resolution and identification of *sec.*- and *tert.*-alkyl gallates from their mixtures by employing thin-layer chromatography; even two-dimensional thin-layer chromatography was not helpful. This was due to the very close proximity of R_F values of branched-chain alkyl gallates, superimposing each other.

TABLE I

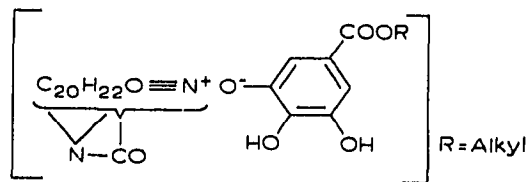
THE R_F VALUES OF VARIOUS GALLATE-ALKALOID MOLECULAR COMPLEXES

Sam- ple No.	Complexes with cinchonine							
	Complexes of cincho- nine-strychnine with the following gallates	Adsor- bents	Kieselgel G impregnated with 2% cinchonine			Magnesium silicate impregnated with 2% cinchonine		
		Solvents	Chloro- form- acetic acid (85:15)	Carbon- tetra- chloride- methyl alcohol (4:1)	Benzene- acetic acid (4:1)	Ethylene- dichlo- ride- methyl alcohol (9:1)	Ethylene- dichlo- ride- acetic acid (9:1)	Chlorofo- rformic acid (9:1)
1	Methyl gallate		0.26	0.23	0.15	0.04	0.22	0.51
2	Ethyl gallate		0.31	0.25	0.21	0.07	0.41	0.83
3	<i>n</i> -Propyl gallate		0.38	0.27	0.24	0.09	0.56	0.87
4	Isopropyl gallate		0.25	0.06	0.08	0.00	0.00	0.00
5	<i>n</i> -Butyl gallate		0.47	0.30	0.26	0.12	0.61	0.89
6	<i>sec.</i> -Butyl gallate		0.19	0.12	0.07	0.00	0.00	0.00
7	<i>tert.</i> -Butyl gallate		0.14	0.16	0.06	0.00	0.00	0.00
8	Isoamyl gallate		0.42	0.22	0.28	0.16	0.65	0.93
9	<i>n</i> -Octyl gallate		0.55	0.34	0.35	0.23	0.93	0.94
10	<i>sec.</i> -Octyl gallate		0.09	0.12	0.06	0.00	0.00	0.04
11	<i>n</i> -Dodecyl gallate		0.68	0.36	0.39	0.29	1.00	0.97

It was observed by the present authors that both cinchonine and strychnine invariably formed 1:1 molecular complexes with alkyl gallates *viz.* methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *sec.*-butyl, *tert.*-butyl, isoamyl, *n*-octyl, *sec.*-octyl and *n*-dodecyl gallate. This was confirmed by varying the gallate-alkaloid ratios and by recording their UV absorption spectra in ethanol.



Cinchonine-alkyl gallate complex.



Strychnine-alkyl gallate complex.

In literature no work on the molecular complexes of alkyl gallates with alkaloids using any of the chromatographic techniques is reported. The present paper describes the resolution and characterization of alkyl gallates up to 2 μ g as their molecular complexes with cinchonine and strychnine employing a thin-layer chromatographic technique. The gallates could be conveniently estimated by measuring the percentage transmission of the eluted spots at 272 m μ .

Complexes with strychnine

<i>Kieselgel G-Kieselguhr</i>) impregnated with <i>strychnine</i>		<i>Kieselgel G impregnated</i> with 2% <i>strychnine</i>			<i>Magnesium silicate im-</i> <i>pregnated with 2% strychnine</i>			<i>Kieselgel G-Kieselguhr G</i> (75:25) impregnated with 2% <i>strychnine</i>		
<i>Ethyl-</i> <i>enedi-</i> <i>-chloride-</i> <i>acetic</i> <i>acid</i> (4:1)	<i>Chloro-</i> <i>form-</i> <i>acetic</i> <i>acid</i> (9:1)	<i>Chloro-</i> <i>form-</i> <i>acetic</i> <i>acid</i> (4:1)	<i>Chloro-</i> <i>form-</i> <i>methyl</i> <i>alcohol</i> <i>acid</i> (9:1)	<i>Benzene-</i> <i>methyl</i> <i>alcohol-</i> <i>acetic</i> <i>acid</i> (45:8:4)	<i>Chloro-</i> <i>form-</i> <i>acetic</i> <i>acid</i> (4:1)	<i>Chloro-</i> <i>form-</i> <i>acetic</i> <i>acid</i> (4:1)	<i>Benzene-</i> <i>acetic</i> <i>acid</i> (9:1)	<i>Chloro-</i> <i>form-</i> <i>methyl</i> <i>alcohol</i> (9:1)	<i>Chloro-</i> <i>form-</i> <i>acetic</i> <i>acid</i> (9:1)	<i>Carbon-</i> <i>tetra-</i> <i>chloride-</i> <i>methyl</i> <i>alcohol</i> (7:3)
0.49	0.15	0.24	0.31	0.18	0.19	0.55	0.09	0.48	0.07	0.45
0.64	0.24	0.32	0.34	0.21	0.24	0.85	0.22	0.52	0.12	0.50
0.80	0.31	0.41	0.39	0.25	0.27	0.90	0.32	0.55	0.15	0.52
0.19	0.03	0.04	0.09	0.15	0.00	0.06	0.00	0.16	0.03	0.40
0.86	0.35	0.49	0.40	0.35	0.29	0.90	0.40	0.56	0.18	0.52
0.21	0.03	0.03	0.11	0.17	0.00	0.05	0.00	0.17	0.04	0.38
0.22	0.02	0.06	0.10	0.18	0.00	0.05	0.00	0.18	0.02	0.39
0.91	0.38	0.55	0.40	0.40	0.33	0.90	0.42	0.57	0.23	0.47
0.91	0.48	0.66	0.46	0.51	0.41	0.92	0.94	0.65	0.25	0.54
0.20	0.03	0.05	0.08	0.14	0.00	0.07	0.00	0.16	0.03	0.35
0.94	0.53	1.00	0.56	0.60	0.50	0.94	1.00	0.76	0.29	0.57

EXPERIMENTAL

Preparation of alkyl gallates

Gallates of methyl, ethyl, propyl, isopropyl, *n*-butyl, *sec.*-butyl, *tert.*-butyl and isoamyl alcohols were prepared by refluxing a mixture of dry gallic acid (1 mole), alcohol (5 moles), toluene (10 moles), *p*-toluene sulfonic acid (0.05 moles) and sulfuric acid (0.5 mole), respectively, for 5 h in a heating mantle. After distillation of the solvent and excess alcohol under vacuum, the mass was extracted twice with ether and washed with cold water. The solution was dried over anhydrous magnesium sulfate, the solvent distilled off and the ester crystallized from ethanol (yields 68–72%). The gallates of *n*-octyl, *sec.*-octyl and dodecyl alcohols were prepared by converting gallic acid into galloyl chloride by treatment with thionyl chloride. Alcohol (1 mole), galloyl chloride (1.1 moles) and pyridine (5 moles) were kept on the water bath for 2 h with frequent stirring and were poured over crushed ice. The crystalline mass was filtered and washed with a minimum amount of cold water. The gallates were crystallized as needles from benzene with yields between 62–65%. Their melting points were as follows: methyl gallate, 200°; ethyl gallate, 160°; *n*-propyl gallate, 148°; isopropyl gallate, 123–124°; *n*-butyl gallate, 135°; *sec.*-butyl gallate, 259°; *tert.*-butyl gallate, 248°; isoamyl gallate, 143°; *n*-octyl gallate, 95–96°; *sec.*-octyl gallate, 170°; dodecyl gallate, 97°. The purity of the compounds was established by twodimensional reversed-phase paper chromatography in which single spots were obtained.

Preparation, spotting and irrigation of plates

Kodak photographic glass plates, 22 × 22 cm, were used. Thin films on the plates were prepared as follows. A fine slurry of the adsorbent (30 g) in ethanol–water (1:1, 60 ml) was prepared; it was poured on the glass plates which were tilted from

side to side. The plates were dried overnight at room temperature and activated at 120° for half an hour. The plates were weighed, and the average coating of the adsorbents was recorded. The thin-layer coatings in the cases of Kieselgel G, magnesium silicate and Kieselgel G-Kieselguhr G (3:1) were 4.32, 7.11 and 3.82 mg/cm², respectively.

The plates were impregnated with cinchonine and strychnine by irrigating them with their respective 2% chloroform solutions. The alkaloid-coated plates, after evaporation of the solvent, were immediately spotted with 2 μ g of each ester with a standard microcapillary. The plates were irrigated at $24 \pm 1^\circ$ with appropriate solvents, using an ascending system. The gallate-alkaloid complexes were light gray in color and could be easily located. Fig. 1 is a typical chromatoplate showing the resolutions of complexes of gallates with cinchonine. The R_F values are given in Table I.

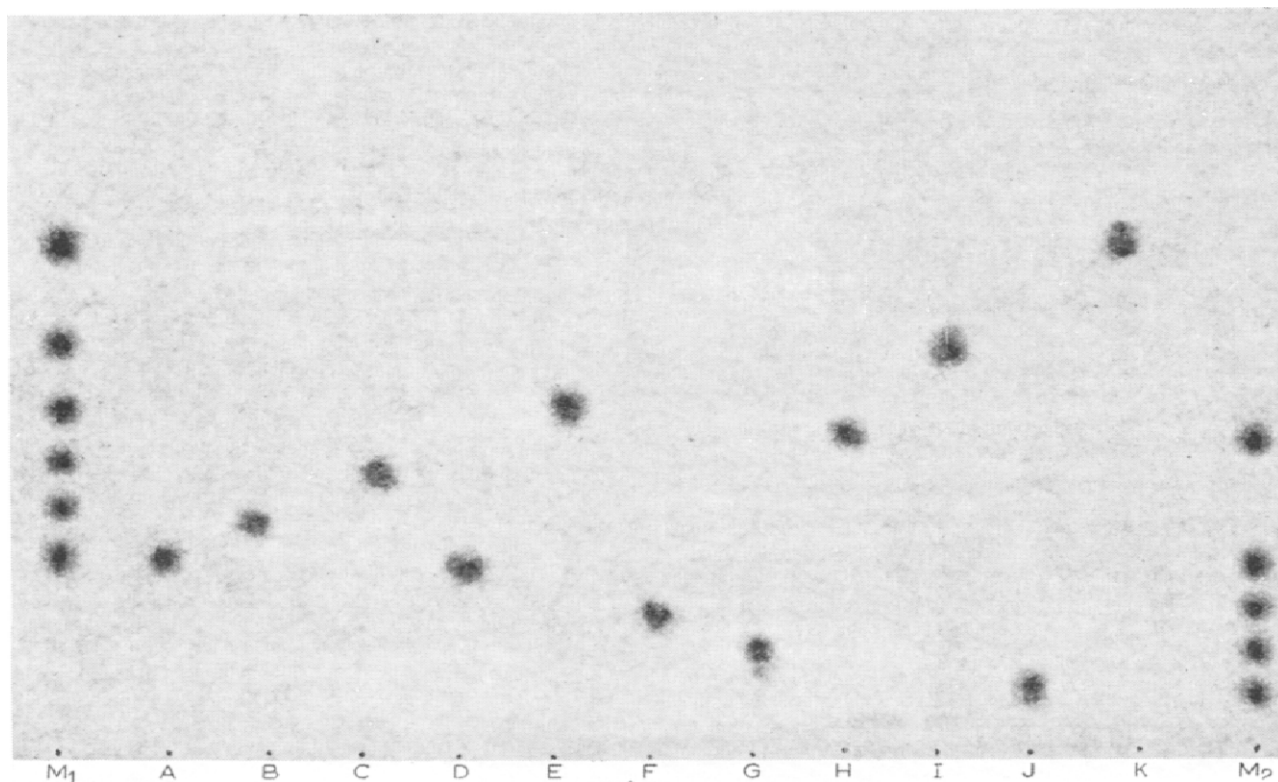


Fig. 1. Thin-layer chromatogram showing the resolutions of molecular complexes of alkyl gallates with cinchonine (Ci). A = methyl gallate-Ci; B = ethyl gallate-Ci; C = propyl gallate-Ci; D = isopropyl gallate-Ci; E = *n*-butyl gallate-Ci; F = *sec.*-butyl gallate-Ci; G = *tert.*-butyl gallate-Ci; H = isoamyl gallate-Ci; I = *n*-octyl gallate-Ci; J = *sec.*-octyl gallate-Ci; K = *n*-dodecyl gallate-Ci; M₁ = mixture of A, B, C, E, I and K; M₂ = mixture of J, G, F, D and H. Adsorbent: Kieselgel G containing 2% cinchonine. Solvent: chloroform-acetic acid (85:15) with 2% cinchonine; system: ascending.

Quantitative estimation of alkyl gallates

The spots of known and unknown quantities of gallates were separately scrapped with a microspatula and extracted with a known volume of 90% ethanol. The percentage transmission of each solution was recorded at 272 $m\mu$, and the quantity of the gallates was calculated.

Discussion and results

When the alkyl gallates with their mixture were run on untreated Kieselgel G and magnesium silicate plates employing different solvent systems, very close R_F values, overlapping each other particularly in the cases of *sec.*- and *tert.*-alkyl gallates, resulted. It was not possible to separate these branched-chain alkyl gallates even by two-dimensional thin-layer chromatography. The typical R_F values of various gallates on untreated plates were as follows:

Adsorbent Kieselgel G; solvent system: petroleum ether 60–80°–acetic acid (5:1); A, 0.16; B, 0.18; C, 0.21; D, 0.14; E, 0.26; F, 0.13; G, 0.12; H, 0.25; I, 0.30; J, 0.13; K, 0.39.

Adsorbent magnesium silicate; solvent system: carbon tetrachloride–acetic acid (10:1); A, 0.08; B, 0.10; C, 0.11; D, 0.06; E, 0.13; F, 0.06; G, 0.05; H, 0.13; I, 0.21; J, 0.06; K, 0.26.

Kieselgel G and Kieselgel G–Kieselguhr G (3:1) proved to be excellent adsorbents for the complexes studied. Incorporation of Kieselguhr G greatly helped in the migration of complexes. The spots were quite distinct in both cases, and there was no tailing. Magnesium silicate required more time for irrigation and was not helpful in good resolutions of isopropyl gallate, *sec.*-butyl gallate, *tert.*-butyl gallate and *sec.*-octyl gallate complexes.

Polarity of solvents played an important role. Higher polarity favored increase in the movement of complexes and hence better resolutions of mixtures.

In general the R_F values of the complexes of alkyl gallates containing normal alkyl groups increased with the rise in their hydrocarbon radicals. Molecular complexes of esters having branched-chain alkyl groups with both cinchonine and strychnine had lower mobilities compared to their straight-chain analogues. This apparently showed a steric influence of the branched-chain molecular complexes on their migrations. The alkyl gallates could be conveniently characterized and estimated up to 2 μg .

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