Reinvestigation of the Biological Activities of Diacetylapomorphine and "Triacetylapomorphine"

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Diacetylapomorphine has been shown to possess emetic activity nearly identical to that of apomorphine itself, corroborating findings of some earlier workers and contradicting certain others. "Triacetylapomorphine" has been demonstrated to be completely nonemetic (in agreement with the original literature report) and to possess moderate hypotensive properties.

DIACETYLAPOMORPHINE and "triacetylapomorphine" were first prepared by Tiffeneau and Porcher (1) in 1915. "Triacetylapomorphine," an amide resulting from ring cleavage of diacetylapomorphine, was reported as being devoid of emetic activity.[1] In a series of papers, Tiffeneau first reported that diacetylapomorphine was equally as potent an emetic as apomorphine (2); later, that it was twice as potent (3). Tiffeneau's explanation for this high emetic activity of diacetylapomorphine was that it is rapidly hydrolyzed in the body to free apomorphine. However, the experiments of Eddy (4) tend to contradict this hypothesis; this worker demonstrated that, in compounds related to morphine, masking of hydroxyls by alkyl or acetyl functions eliminated central emetic activity.[2] Of particular significance is 3,4-dihydroxyphenanthrene, which is structurally similar to apomorphine and is a moderately potent emetic; diacetylation renders this molecule nonemetic.[2] Since the work of Tiffeneau is rather old, and since his various publications contradict Eddy's reports and each other, it seemed advisable to reinvestigate the pharmacological properties of the acetylation products of apomorphine.

"Triacetylapomorphine" was prepared by heating apomorphine hydrochloride in acetic anhydride according to the method of Tiffeneau (1). It displayed no emetic activity when administered subcutaneously to dogs in a dose range up to 100 times the dependable emetic dose of apomorphine. It did not elicit the characteristic pecking syndrome in pigeons (5), and it exhibited no antiacetylcholinesterase activity. However, it showed moderate hypotensive activity.

Diacetylapomorphine was prepared in 10% yield by treating apomorphine hydrochloride with acetic anhydride and pyridine at room temperature according to the method of Small (6), using Tiffeneau's procedure (1) for recrystallization. This compound appeared to be identical to apomorphine in regard to minimal emetic dose and time of onset of action, although the severity of vomiting is of a lower order. It is a potent pecking syndrome stimulant in the pigeon.

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[1] The abstract of *Reference* 2, as reported in *Chem. Abstr.*, 8, 3191(1914), incorrectly states that "triacetylapomorphine" is identical to apomorphine in its physiological properties.

[2] While the acetylated and alkylated compounds often induce symptoms of nausea in the cat (as indicated by excessive salivation and licking), they are considered here as being nonemetic since they did not cause actual vomiting.

TABLE I.—RESULTS OF BLOOD PRESSURE LOWERING EXPERIMENT

Min. After Injection	B.p., mm.	Respirations/ Min.
0	80	21
0.5	80	20
1	82	23
3	66	28
5	45	25
10	37	25
15	30	22
20	32	24
30	29	24

EXPERIMENTAL

"Triacetylapomorphine" was triturated in a few drops of polysorbate 80[3] and was suspended in 50% propylene glycol solution. For the emesis test, 0.1 to 10 mg./Kg. was administered subcutaneously to six dogs, from whom food had been withheld for 16 hours; however, 15 minutes prior to the injection, the dogs were given about 0.25 lb. of food, an amount insufficient to allay hunger, but enough to provide some bulk in the stomach in the event that vomiting were elicited. During an 8-hour period, there was no evidence of emesis or nausea; that is, there was no salivation, and appetite remained.

For the blood pressure lowering experiment, 4 mg./Kg. of "triacetylapomorphine" was administered intravenously to a 10-Kg. dog, anesthetized with sodium pentobarbital and previously tested for tone and autonomic dominance of peripheral blood vessels in the following manner: Carotid occlusion gave a substantial rise in blood pressure; intravenous injection of 1.5 mcg./Kg. of epinephrine hydrochloride brought about a 25-mm. rise in blood pressure and respiratory depression; intravenous injection of 0.5 mcg./Kg. of methacholine chloride resulted in a 35-mm. drop in blood pressure and mild hyperpnea. The results of the experiment are recorded in Table I.

Carotid occlusion reflex was tested and epinephrine injected at intervals; carotid reflex was depressed but not abolished, and epinephrine response was unaltered.

Diacetylapomorphine was dissolved in a small amount of 0.1 *N* HCl, and was administered in solution, buffered to pH 5.5 with MacIlvain's phosphate-citrate buffer. Four dogs received doses of 0.1 to 0.2 mg./Kg. subcutaneously. For comparison, two dogs received apomorphine hydrochloride, 0.1 and 0.2 mg./Kg., respectively. Emesis occurred within 5 minutes in all cases.

[3] Marketed as Tween 80 by Atlas Powder Co., Wilmington, Del.

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Communications

Effect of Some Perfume Oils on Dehydrogenases in *Escherichia coli*

Sir:

Since the discovery that perfume oils inhibit the growth of bacteria (1, 2) we have investigated the antimicrobial properties of many such oils further (3, 4). The mode of action by which bacteria are killed by perfume oils remains unknown but Gal'perin and Dunaeva (5, 6) have reported that essential oils affect dehydrogenases in paramecia and helminths. Since essential oils are commonly used in perfume oil formulas it was thought that bacterial dehydrogenases might be affected by perfume oils.

We selected as the test organism *Escherichia coli* ATCC 11229. The bacteria were harvested after 24 hours from nutrient agar slants and washed three times with sterile physiological saline by centrifugation. A 2-ml. quantity of washed packed cells was added to 3 ml. of 1:1000 perfume oil solution (1% alcoholic). After 30 minutes of contact the cells were removed by centrifugation and washed three times with sterile saline and suspended in saline to 300 Klett units in the Klett-Summerson photoelectric colorimeter using the No. 42 filter with a spectral range of 400 to 465 mμ. This treated standard cell suspension was used for all subsequent experiments.

The dehydrogenase activity was determined by the Thunberg technique (7). The substrates used were glucose, succinic acid, and malic acid. The color intensity of the methylene blue in the Thunberg tubes was recorded every 4 minutes for a total of 32 minutes. All experiments were run at least in duplicate with controls and were replicated several days later.

Perfume oil of bouquet No. 821, sweet grass, lilac water, and bouquet No. 21 completely inhibited glucose, succinic acid, and malic acid dehydrogenase. Plate counts of the standard cell suspension showed no reduction in cell number compared to controls. However, after 2-hour exposure of the test organism to each perfume oil, plate counts revealed a 10 to 50% reduction depending upon the oil used. This finding suggests that perhaps the reduction of dehydrogenases precedes the death of the cell.

E. coli was exposed to perfume oil of neroli (artificial), evergreen bouquet, osheana, and chypre 66D at a concentration of 1:2000 in the same manner as above and dehydrogenase activity measured. It was found that oil of neroli and evergreen bouquet completely inhibited all tested dehydrogenases, while oil of osheana and chypre 66D caused little effect on glucose dehydrogenase, more than 90% inhibition of succinic dehydrogenase, and complete suppression of malic dehydrogenase.

Essential oils and their components are presently being investigated for their effects on bacterial dehydrogenases.

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