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The analgesic properties of some 14-substituted derivatives of codeine and codeinone

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The effects of 14-hydroxylation and subsequent 14-acylation on the toxicity and analgesic activity of codeine, codeine-6-acetate, codeinone, and Δ^7-deoxycodeine have been examined in rats and mice. Acute toxicity was reduced in each instance by the introduction of a 14-hydroxy group and was not generally enhanced by its esterification. 14-Acetoxycodeine was approximately equal to morphine in potency and esterification at the 14-position of hydroxycodeine with other straight chain aliphatic acids containing up to 5 carbon atoms failed to enhance analgesic potency further. 14-Benzoylation of either 14-hydroxycodeine or 14-hydroxycodeinone had little effect on analgesic activity but the introduction of a methylene group between the carboxyl group and the phenyl ring enhanced potency considerably in each case. Increasing the number of carbon atoms from 2 to 5 in the 14-acyl groups of esters of 14-hydroxycodeinone and 14-hydroxy-Δ^7-deoxycodeine led to a gradual increase in analgesic activity. In rats the n-valeryl ester of 14-hydroxy-Δ^7-deoxycodeine was estimated to have 75 times the potency of morphine.

THE properties of morphine derivatives are modified by the introduction of a hydroxyl group at position 14. Reduction of acute toxicity to mice occurs with dihydrocodeine (Winder, Jones, Weston & Gajewski, 1959), dihydrocodeinone (Krueger, Eddy & Sumwalt, 1943) and dihydromorphinone (Blumberg, Carson & Stein, 1954). The effect on analgesic properties is variable; the potencies of dihydrocodeinone (Krueger, Eddy & Sumwalt, 1943) and codeine (Sargent, Schwartzman & Small, 1958) remain unchanged whilst that of dihydromorphinone (Blumberg & others, 1954) is slightly enhanced.

There is little information on effects resulting from acylating the 14-hydroxyl group; Sargent & others (1958) described only the preparation of 14-acetoxycodeine-6-acetate, but 14-acetoxycodeinone is less toxic and slightly more potent as an analgesic than 14-hydroxycodeinone (Krueger & others, 1943).

The availability of compounds listed in Table 1 (Currie, Gillon, Newbold & Spring, 1960) enabled us to study systematically the effects of both hydroxylation and subsequent acylation at the 14-position on the toxicity and analgesic properties of codeine (series I), codeine-6-acetate (series II), codeinone (series III) and Δ^7-deoxycodeine (series IV).

Methods

ACUTE TOXICITY IN MICE

The subcutaneous LD50 of each compound was determined using albino mice weighing between 18–22 g. Compounds were dissolved in 0·9% w/v sodium chloride and administered in a volume of 0·2 ml/20 g body weight. Mortalities were recorded 24 hr later.

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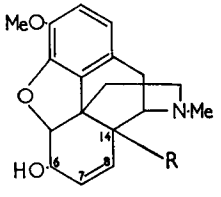
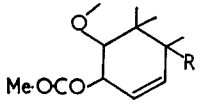
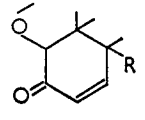
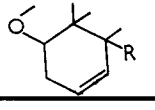
ANALGESIA IN RATS

Analgesia was determined by a modification of the method described by Green & Young (1951), in which pressure is applied to the tail by the plunger of a hypodermic syringe. The barrel of the syringe is connected to a reservoir almost completely filled with an aqueous solution of glycerol 50% v/v connected to a source of compressed air. Normally the air is allowed to leak away through a small hole at the top of the reservoir but when the hole is closed with the finger the rise in pressure forced the plunger of the syringe downwards on to the tail. Inflow of air is adjusted so that the pressure within the reservoir rose at a rate of approximately 2 cm Hg/sec.

TABLE 1. COMPOUNDS INVESTIGATED

Series	No.	Name
I 	1	Codeine phosphate
	2	14-Hydroxycodeine hydrochloride
	3	14-Acetoxycodeine hydrochloride
	4	14-Propionoxycodeine hydrochloride
	5	14-n-Butyryloxycodeine hydrochloride
	6	14-n-Valeryloxycodeine hydrochloride
	7	14-Benzoyloxycodeine hydrochloride
	8	14-Phenylacetoxycodeine hydrochloride
	9	14-Nicotinoyloxycodeine dihydrochloride
II 	10	Acetylcodeine acid tartrate
	11	14-Hydroxycodeine-6-acetate hydrochloride
	12	14-Acetoxycodeine-6-acetate
	13	14-Propionoxycodeine-6-acetate hydrochloride
	14	14-Benzoyloxycodeine-6-acetate hydrochloride
III 	15	Codeinone
	16	14-Hydroxycodeinone
	17	14-Propionoxycodeinone
	18	14-n-Butyryloxycodeinone
	19	14-n-Valeryloxycodeinone
	20	14-Phenylacetoxycodeinone
	21	14-Nicotinoyloxycodeinone
IV 	22	14-Hydroxy-Δ^7-deoxycodeine hydrochloride
	23	14-Acetoxy-Δ^7-deoxycodeine
	24	14-n-Butyryloxy-Δ^7-deoxycodeine
	25	14-n-Valeryloxy-Δ^7-deoxycodeine

The analgesic effect of compounds was determined 30 min after subcutaneous injection of drug in normal saline or 60 min after oral administration of a solution in water. Control animals received the appropriate solvent only. In all estimates of potency at least three dose levels of drug were employed and compounds were compared with morphine on the same day.

Female Wistar rats weighing between 30 and 60 g were distributed randomly into groups of 10. The mean pressure required to elicit a squeak was determined for control animals and the proportion of animals in each treated group which failed to respond at twice this pressure was recorded.

ANALGESIA IN MICE

Estimates of analgesic activity were made using the method of Bianchi & Franceschini (1954). Albino mice weighing 18–22 g were tested for sensitivity to a bulldog artery clip covered with rubber tubing, which was applied to the base of the tail and only those attempting to remove the clip within 15 sec were used. Each compound was administered subcutaneously at three of four dose levels to groups of 10 animals. Animals were tested 30 min later and the proportion in each group which made no attempt to remove the clip within 30 sec was determined.

RESPIRATORY RATE

The respiratory rate in unanaesthetised Wistar rats weighing 40–50 g was determined before and 30 min after subcutaneous injection of drug solution. Groups of five animals were used at each dose level, the animals being loosely restricted in a rigid plastic tube attached to a membrane micromanometer (Infra Red Development Co.) and pen recorder. The dose needed to reduce the respiratory rate by 50% was estimated graphically.

GASTROINTESTINAL EFFECTS

The influence of drugs on intestinal motility in mice was studied using groups of ten animals isolated in individual cages with a wire mesh floor. The faecal weight of drug-treated groups was compared with that of a control group receiving normal saline. The percentage reduction in faecal weight obtained during 2 hr after subcutaneous administration of drug was determined, and relative potency calculated quantally with respect to morphine.

The antagonism of the compounds to acetylcholine, histamine and barium chloride on the isolated guinea-pig ileum was studied using the superfusion method of Adam, Hardwick & Spencer (1954).

Calculations of the LD50, the ED50 and relative potency in experiments giving quantal results were carried out by the method of Litchfield & Wilcoxon (1949).

Results

ACUTE TOXICITY

Death generally occurred within 3 hr after subcutaneous administration, and appeared to be due to respiratory failure. Except in the instance of codeinone and 14-hydroxycodeinone, which were convulsants, the symptoms at near toxic dose levels consisted of catalepsy without loss of righting reflex and acute respiratory depression. The LD50 was determined from mortalities recorded 24 hr after drug administration (Table 2).

Hydroxyl substitution at position 14 caused more than a fivefold reduction in the acute toxicity of codeine or codeine-6-acetate and the effect on Δ^7-deoxycodeine appeared to be similar. No estimate of the LD50 of Δ^7-deoxycodeine was obtained in the present work but the calculated subcutaneous LD50 of the base in mice determined by Karrer

& Widmark (1951) was 60 mg/kg compared with 700 mg/kg for 14-hydroxy-Δ^7-deoxycodeine. The introduction of a 14-hydroxy group into the codeinone molecule was much less effective, the LD50 increasing only from 11 to 28 mg/kg.

Esterification of the 14-hydroxyl group did not produce marked changes in the acute toxicity of 14-hydroxycodeine. When esters of the following acids were examined; nicotinic, benzoic, phenylacetic and the homologous series from acetic to valeric the range of LD50s determined varied only from 400 to 640 mg/kg. Similar results were obtained with

TABLE 2. THE ACUTE TOXICITY OF CODEINES AND CODEINONES IN ALBINO MICE AFTER SUBCUTANEOUS ADMINISTRATION

[All values are calculated in terms of anhydrous base. Limits of error (P = 0·95) shown in parentheses]

	Codeine derivatives		Codeine-6-acetate derivatives		Codeinone derivatives		Δ^7-Deoxycodeine derivatives	
14-Substituent	Compound No.	LD50 mg/kg	Compound No.	LD50 mg/kg	Compound No.	LD50 mg/kg	Compound No.	LD50 mg/kg
None	1	120 (100–150)	10	140 (120–170)	15	11·0 (9·8–12·3)		60†
-OH	2	880 (730–1050)	11	760 (570–1000)	16	28 (25–30)	22	700 (600–830)
-OCOMe	3	560 (480–660)	12	630 (530–750)		127*	23	>500
-OCOEt	4	420 (360–470)	13	430 (340–560)	17	150 (110–190)		
-OCOPr	5	640 (580–750)					24	180 (160–210)
-OCOBu	6	490 (390–610)			19	495 (410–600)		
-OCO-  ..	7	400 (340–460)	14	>1000				
-OCOCH$_2$-  ..	8	500			20	300		
-OCO-  ..	9	500			21	100–300		

Morphine (estimated as hydrochloride) = 330 (280–390)

† Karrer & Widmark (1951) * Krueger, Eddy & Sumwalt (1943)

the esters of 14-hydroxycodeine-6-acetate examined, but increasing the size of the 14-substituent in the codeinone series reduced toxicity. In the case of 14-hydroxy-Δ^7-deoxycodeine however the results suggest that enhanced toxicity might be expected with increasing length of the alkyl chain.

ANALGESIA

The effects of substitution at position 14 of codeine-6-acetate, codeinone and Δ^7-deoxycodeine on analgesic activity in rats at 30 min after subcutaneous injection are most clearly seen in relation to the results obtained with derivatives of codeine (Table 3). 14-Hydroxycodeine did not differ

in potency from codeine but acylation of the 14-alcoholic hydroxyl group generally enhanced activity. Those derivatives in which the acyl group contained an unbranched alkyl chain equalled morphine in potency but it was clear that the enhanced activity was independent of chain length since increasing the number of carbon atoms in it from one to four did not produce any marked alterations in potency. The presence of an aromatic or heterocyclic ring adjacent to the carbonyl group was not necessarily advantageous since both the benzoyl and the nicotinoyl esters of 14-hydroxycodeine were only slightly more potent than 14-hydroxycodeine itself, but introduction of a single methylene group

TABLE 3. THE ANALGESIC ACTIVITY OF CODEINES AND CODEINONES IN RATS 30 MIN AFTER SUBCUTANEOUS ADMINISTRATION

[The figures represent relative potency (morphine = 1·0) of compounds in terms of base. Limits of error (P = 0·95) are shown in parentheses]

14-Substituent	Compound No.	Codeine derivatives	Compound No.	Codeine-6-acetate derivatives	Compound No.	Codeinone derivatives	Compound No.	Δ^7-Deoxy-codeine derivatives
None	1	0·17 (0·09–0·31)	10	0·13 (0·08–0·23)	15	*		
–OH	2	0·13 (0·07–0·23)	11	0·20 (0·13–0·22)	16	*	22	0·34 (0·21–0·42)
–OCOMe	3	1·3 (0·9–2·4)	12	0·11 (0·06–0·19)			23	2·7 (1·7–4·2)
–OCOEt	4	1·3 (0·8–2·3)	13	1·0 (0·7–1·6)	17	2·5 (1·4–4·7)		
–OCOPr	5	1·7 (1·1–2·7)			18	16 (11–25)	24	7·4 (4·6–12·0)
–OCOBu	6	1·0 (0·6–1·7)			19	66 (41–105)	25	75 (37–152)
–OCO–C$_6$H$_5$ ..	7	0·19 (0·12–0·31)	14	0·05				
–OCOCH$_2$–C$_6$H$_5$ ..	8	3·4 (1·9–5·9)			20	**45** (29–68)		
–OCO–C$_5$H$_4$N ..	9	0·33 (0·21–0·57)			21	0·36 (0·23–0·56)		

* Could not be determined because of toxic excitation

between the carbonyl group and the phenyl ring of 14-benzoyloxycodeine led to a considerable increase in activity.

Similar substitution in codeine-6-acetate did not produce any striking increase in analgesic action. Codeine-6-acetate itself, 14-hydroxycodeine-6-acetate, 14-acetoxycodeine-6-acetate, 14-benzoyloxycodeine-6-acetate and codeine were almost equi-potent but 14-propionoxycodeine-6-acetate equalled morphine in activity.

Extending the chain length of the 14-acyloxy group in the case of codeinone derivatives modified the properties of the resulting compounds considerably. Altering the number of carbon atoms in the straight alkyl chain from two to four increased potency relative to morphine from 2·5 to 66 but, as in the case of codeine derivatives, nicotinoyl substitution was

ineffective whereas phenylacetyl gave a compound estimated to have 45 times the potency of morphine.

Acylation of 14-hydroxy-Δ^7-deoxycodeine by groups containing straight alkyl chains also enhanced analgesic activity. A twentyfive-fold enhancement resulted when the number of carbon atoms in the chain was increased from one to four, 14-n-valeryloxy-Δ^7-deoxycodeine being 75 times as potent as morphine.

It is interesting to note that the minimum number of carbon atoms in the acyloxy group at the 14-position necessary to produce an increase in analgesic potency was not the same in each series. In the case of codeine and Δ^7-deoxycodeine derivatives it occurred with acetoxy and with codeine-6-acetate and codeinone with propionoxy substitution.

Those compounds tested 60 min after oral administration generally exhibited similar potency to that obtained 30 min after subcutaneous injection (Table 4) indicating good intestinal absorption.

TABLE 4. THE ANALGESIC ACTIVITY OF CODEINES AND CODEINONES IN RATS 1 HR AFTER ORAL ADMINISTRATION

[The figures represent relative potency (morphine = 1·0) of compounds in terms of base. Limits of error (P = 0·95) are given in parentheses]

14-Substituent	Compound No.	Codeine derivatives	Compound No.	Codeinone derivatives
-OCOMe	3	0·5 (0·2–1·1)		
-OCOEt	4	0·7 (0·4–1·2)		
-OCOPr	5	1·8 (1·0–3·4)	18	59 (24–117)
-OCOBu	6	0·9 (0·4–2·0)	19	59 (31–114)
-OCOCH$_2$-  ..			20	60 (33–111)

Results obtained after subcutaneous injection in mice are given in Table 5. The potencies of compounds relative to morphine were generally estimated to be less than those found in rats by the same route and the length of the alkyl chain in the 14-position necessary to obtain a large increase in potency was greater. Thus in the codeine series acetylation of the 14-hydroxy group gave a ten-fold increase in potency in rats but no change in mice and an approximate five-fold increase was obtained only with the 14-propionic ester. Similarly on esterifying 14-hydroxycodeine-6-acetate with propionic acid the marked increase in potency found in rats was not observed with mice. The 14-n-valeryloxy derivatives of codeinone and Δ^7-deoxycodeine were much less analgesic in mice.

RESPIRATORY RATE

14-n-Butyryloxycodeine was compared with morphine for its effect on respiratory rate in unanaesthetised rats. A linear relationship was obtained between log dose and percentage reduction in rate. It produced

relatively greater depression of the respiratory rate than an equi-analgesic dose of morphine (Table 6). The respiratory depression could be reversed by nalorphine or levallorphan.

CARDIOVASCULAR SYSTEM

14-n-Butyryloxycodeine, 1 mg/kg, produced a transient fall in blood pressure when injected intravenously into dogs anaesthetised with pentobarbitone in contrast to the more prolonged fall obtained after morphine,

TABLE 5. THE ANALGESIC ACTIVITY OF CODEINES AND CODEINONES IN MICE AFTER SUBCUTANEOUS ADMINISTRATION

[The figures represent relative potency (morphine = 1·0) of compounds in terms of base. *Limits of error* (P = 0·95) *are given in parentheses*]

14-Substituent	Compound No.	Codeine derivatives	Compound No.	Codeine-6-acetate derivatives	Compound No.	Codeinone derivatives	Compound No.	Δ²-Deoxycodeine derivatives
-OH	2	0·12 (0·05–0·28)	11	0·35 (0·20–0·62)	16	*	22	0·34 (0·18–0·62)
-OCOMe	3	0·11 (0·04–0·29)				About ½ potency of codeine†	23	0·88 (0·45–1·73)
-OCOEt	4	0·7 (0·3–1·5)	13	0·41 (0·25–0·66)				
-OCOPr	5	0·6 (0·3–1·2)						
-OCOBu	6	0·47 (0·24–0·92)			19	7·8 (3·7–16)	25	4·8 (2·5–9·2)
-OCOCH₂  ..	8	7·7 (4·2–14·4)			20	26 (13–52)		
-OCO-  ..	9	0·32 (0·17–0·61)			21	0·15 (0·07–0·29)		

* Could not be determined because of toxic excitation
† Sargent, Schwartzman & Small (1958)

TABLE 6. THE EFFECT OF 14-n-BUTYRYLOXYCODEINE ON RESPIRATORY RATE IN RATS AND GASTROINTESTINAL MOTILITY IN MICE

[Values are expressed in terms of base. Limits of error (P = 0·95) are shown in parentheses]

Compound	Respiratory rate		*Gastrointestinal motility*	
	ED50 mg/kg s.c.	Relative potency	ED50 mg/kg s.c.	Relative potency
Morphine hydrochloride	15	1	1·5 (1·0–2·1)	1
14-n-Butyryloxycodeine hydrochloride	4·9	2·6	5·1 (3·3–7·9)	0·3

1 mg/kg. Rapid tolerance was acquired to both drugs, the third successive dose in each case failing to produce any effect on the blood pressure. No changes in the electrocardiogram record were observed, nor were the normotensive responses to adrenaline, acetylcholine, histamine, carotid occlusion and vagal stimulation affected in any way.

GASTROINTESTINAL EFFECTS

The stimulant actions of acetylcholine (5×10^{-8}), histamine (5×10^{-8}) and barium chloride (2×10^{-5}) were not antagonised by any of these derivatives given in a concentration of 10 μg/ml. However 14-n-butyryloxycodeine exhibited a constipating activity in mice (Table 6), its relative potency compared with morphine being similar to its analgesic potency in mice.

Discussion

The results extend the previous observations of Krueger & others (1943), Blumberg & others (1954), and Winder & others (1959), that substitution of the hydroxyl group at position 14 in alkaloids of the morphine group usually reduces acute toxicity. This action was most marked in the case of codeine and Δ^7-deoxycodeine where hydroxylation resulted in more than a five-fold lowering of acute toxicity in mice. It was much less so in the case of codeinone where the subcutaneous LD50 was only increased from 11 mg/kg to 28 mg/kg.

Winder & others (1959) have discussed the importance of the 6-position of morphine derivatives in influencing toxicity and stress that any interference with the alcoholic function at this point is likely to enhance toxic excitation. Such increased toxicity was found with codeinone and 14-hydroxycodeinone, but was absent or much attenuated in the instances of codeine-6-acetate and Δ^7-deoxycodeine and their 14-hydroxy derivatives. The reduction of toxicity induced by the 14-hydroxy group was maintained after its esterification and with 14-hydroxycodeinone this esterification further reduced toxicity. Thus acylation of 14-hydroxycodeinone to give 14-n-valeryloxycodeinone increased analgesic potency more than a hundred-fold and reduced acute toxicity by a factor of more than a hundred. This change in acute toxicity is unlikely to be of much significance and may only relate to a change from stimulant to depressant properties in mice.

Our results indicate that the effects of esterifying the 14-hydroxyl group of codeine and related compounds depends upon both the nature of the esterifying acid and upon the groups present at positions 6, 7 and 8 of the molecule. Analgesic potency is mainly unaffected by acetylating the 6-hydroxy group (Table 1, series II) but greatly enhanced by oxidising it to a ketone (series III) or by removing all substituents at position 6 (series IV). The general observation of Braenden, Eddy & Halbach (1955), that a free alcoholic hydroxyl at position 6 interferes with analgesia in the morphine group is supported by the failure to obtain potent analgesics in the codeine series similar to those obtained from codeinone and Δ^7-deoxycodeine.

Preliminary results suggest that 14-n-butyryloxycodeine has no obvious advantages over morphine. The greater potency of codeinone and Δ^7-deoxycodeine derivatives is of interest in attempting to relate structure to analgesic activity but such compounds have no advantages over those

in current clinical use unless their analgesic properties can be dissociated from undesirable effects.

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