Tritium labelling of 2-(O-Chlorophenyl)-2-(Methylamino)-Cyclohexanone (Ketamine; CI-581) by acid catalyzed exchange: an example of intramolecular competition for Tritium between substituted aromatic and cyclohexanone rings

C. E. BLACKBURN and R. E. OBER *
Research Division, Parke, Davis and Company
Ann Arbor, Michigan, U.S.A.

Received on 9th January 1967

SUMMARY

The preparation and characterization of tritium-labelled 2-(o-chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride is described. Under the exchange conditions employed (CF_3COOH, 10 c ^3H_2O, Pt), it was expected that the tritium would be distributed between the phenyl and cyclohexanone rings. Our observations indicated that the major portion of the tritium was located in the cyclohexanone ring.

INTRODUCTION.

The pharmacological properties of 2-(*o*-chlorophenyl)-2-(methylamino)-cyclohexanone hydrochloride, hereinafter referred to as CI-581·HCl, have recently been described by McCarthy *et al.* [1]. This paper describes the preparation and characterization of tritium labelled CI-581·HCl which will be used to extend the metabolic studies reported earlier by Chang *et al.* [2].

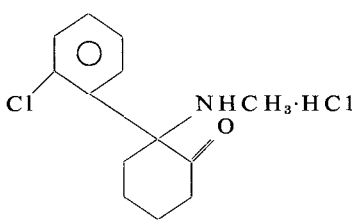


Fig. 1. CI-581·HCl

* College of Pharmacy, The Ohio State University, Columbus, Ohio, U.S.A.

CI-581·HCl contains both an aromatic ring and a cyclohexanone ring as shown in Figure 1. The preparation of CI-581-^{3}H·HCl by an acid catalyzed exchange procedure provided an interesting example of the relative competition for tritium between the two ring systems.

PREPARATION OF CI-581-^{3}H·HCl.

Preliminary experiments with millicurie quantities of tritium demonstrated that CI-581·HCl could be labelled by acid catalyzed exchange to yield CI-581-^{3}H having a radiochemical purity of >90%. Subsequently, a sample of CI-581·HCl was exchange-labelled as follows * : 50.0 mg of CI-581·HCl was dissolved in 0.2 ml of trifluoroacetic acid containing 10 curies of tritiated water and 25.0 mg of prereduced platinum catalyst. The reaction mixture was heated at 100° C for eighteen hours while being stirred magnetically. The reaction mixture was cooled, diluted by the addition of 10.0 ml 0.0001 *N* HCl, and vacuum distilled to dryness to remove labile tritium. The tritiated residue was dissolved in 10.0 ml 0.0001 *N* HCl. The total incorporated tritium in the product was 146 mc.

A chromatography study ** (TLC) indicated that the tritiated product was CI-581-^{3}H·HCl with a radiochemical purity of approximately 95%. After this product had been stored in 0.01 *N* HCl at —20° C for approximately one year, the free base was extracted into chloroform from 0.1 *N* NaOH. Chromatography studies on the chloroform extract in five TLC systems established that the radiochemical purity of CI-581-^{3}H remained unchanged.

To obtain CI-581-^{3}H of higher radiochemical purity, the chloroform extract was concentrated to approximately 2 ml by evaporation and then chromatographed preparatively on a thin layer plate (Silica Gel G_F, 500 μ thick; Et_2O : MeOH : H_2O, 4 : 4 : 1). CI-581-^{3}H was eluted from the gel with diethyl ether and then filtered through paper to yield 47.9 mc CI-581-^{3}H (free base). A chromatography study (TLC : Silica Gel G_F; Et_2O : MeOH : H_2O, 4 : 4 : 1) of the ether eluate revealed CI-581-^{3}H with a radiochemical purity of 99%.

The high specific activity CI-581-^{3}H (free base, 47.9 mc ^{3}H) in 60 ml ether was diluted by the addition of 1.10 g (4.63 mmoles) non-radioactive CI-581 (free base ***). This ethereal mixture was concentrated by evaporation

* The exchange labelling was performed by New England Nuclear Corporation, Boston, Massachusetts.

** In all of the chromatography studies, the chromatograms were scanned for tritium by analyzing 1 cm and/or 0.5 cm sections using liquid scintillation techniques.

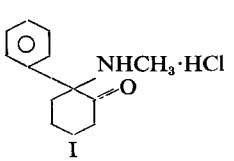
*** Non-radioactive CI-581 (free base) was obtained from CI-581·HCl as follows : CI-581·HCl was dissolved in 0.01 *N* HCl, precipitated with 50% NaOH, filtered, and finally dried to yield CI-581 (free base), m.p. 91-91.5° C.

TABLE 1. Summary of characterization of CI-581-^{3}H·HCl.

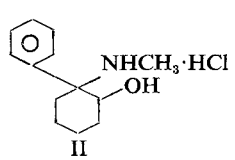
Parameter	CI-581-^{3}H·HCl	Authentic CI-581·HCl
Melting point, °C	259-260 (dec)	259-260 (dec)
Color	White	White
Specific activity (μc/mg)		
CI-581-^{3}H·HCl	34.0 (measured)	—
CI-581-^{3}H (free base)	39.2 (calculated)	—
Elemental analysis		
C calc. 56.94%	56.55% 56.72%	—
H calc. 6.25%	6.32% 6.23%	—
N calc. 5.11%	4.97% —	—
Ultraviolet analysis in 0.1 *N* HCl	λ 276 ε 551	λ 276 ε 562
	269 625	269 636
Chromatography	A major ^{3}H spot having the same R_f as authentic CI-581·HCl was detected in five TLC solvent systems and in one PC solvent system [a]. CI-581 analogues [b] I, II and III were eliminated as possible radiochemical impurities (≤0.1%) since they were separated from CI-581·HCl in at least one of the chromatography systems; IV was detected in amounts of ≤1.4%; a maximum total radiochemical impurity of ≤1.8% was detected.	
Tritium label stability	As discussed in detail in the text, the tritium in CI-581-^{3}H·HCl is stable in aqueous HCl of concentration ≤1.0 *N*; as much as 77% of the tritium is labile in aqueous NaOH.	

[a] CI-581-^{3}H·HCl and the authentic reference compounds were chromatographed on Silica Gel G_F (TLC) or on Whatman No. 1 paper (PC). The following chromatography systems were used: Et_2O : MeOH : H_2O, 4 : 4 : 1 (TLC); $CHCl_3$: EtOH, 1 : 1 (TLC); $CHCl_3$: Acetone : Et_2NH, 5 : 4 : 1 (TLC); Benzene : EtOAc : Et_2NH, 7 : 2 : 1 (TLC); $CHCl_3$: EtOAc : HN_4OH (conc.), 60 : 40 : 1 (TLC); *n*-BuOH saturated with 3% HOAc (PC).

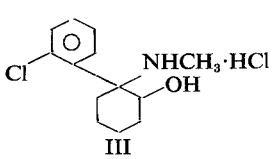
[b] Analogues of CI-581·HCl,



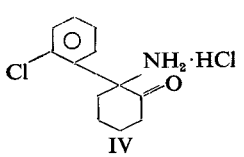
I



II



III



IV

to 40 ml before adding, with stirring, 2 ml isopropyl alcohol and then 1.8 ml isopropyl alcohol saturated with HCl. The resulting precipitate was collected by filtration, washed with diethyl ether and dried at room temperature under vacuum to yield 1.254 g CI-581-^{3}H·HCl with a specific activity of 34.0 μc/mg. The characterization data are summarized in Table I.

TRITIUM LABEL STABILITY OF CI-581-^{3}H·HCl (*in vitro*).

Preliminary experiments which indicated that some of the tritium could be removed from CI-581-^{3}H·HCl under certain conditions in aqueous solution prompted a more extensive tritium stability study. The general experimental approach of this study was as follows : CI-581-^{3}H·HCl was exposed to a range of pH conditions in aqueous solutions for different periods of time and then extracted into chrloroform. The total tritium content of each phase was determined. Large quantities of tritium detected in the aqueous phase after

TABLE 2. Results of the tritium label stability experiments on CI-581-^{3}H·HCl.

Experiment	Exchange conditions [a]			% ^{3}H in [b]	
	Exchange medium	Time	Extraction medium	Chloroform phase	Aqueous phase [c]
1	pH 2 HCl	18 hr	pH 9 NaOH	93.2	2.0
2	pH 2 HCl	1 hr	pH 9 NaOH	92.5	1.2
3	pH 2 HCl	1 hr	pH 9 NaOH	94.9	1.1
1	pH 2 citric acid	18 hr	pH 8 Na_3PO_4	102.1	0.8
2	pH 2 citric acid	1 hr	pH 8 Na_3PO_4	99.9	<0.5
3	pH 2 citric acid	1 hr	pH 8 Na_3PO_4	104.2	1.0
5*a*	0.1 *N* HCl	1 hr	pH 8-8.5 Na_3PO_4	98.5	0.8
5*b*	1.0 *N* HCl	1 hr	pH 8-8.5 Na_3PO_4	100.5	1.1
4*a*	0.1 *N* NaOH	1 hr	0.1 *N* NaOH	52.9	44.3
6	0.1 *N* NaOH	1 hr	0.1 *N* NaOH	65.1	38.8
4*b*	1.0 *N* NaOH	1 hr	1.0 *N* NaOH	27.6	69.3
7	1.0 *N* NaOH	1 hr	1.0 *N* NaOH	29.7	69.1
8	0.1 *N* NaOH	18 hr	0.1 *N* NaOH	27.2	69.2
9	1.0 *N* NaOH	18 hr	1.0 *N* NaOH	23.3	77.1
14	1.0 *N* NaOH	2 days	1.0 *N* NaOH	25.6	76.4

[a] All of the experiments were conducted at room temperature (approximately 25° C) except experiment 2 which was run at 37° C. See Appendix A.

[b] The % ^{3}H in the final separated phases is based on the measured ^{3}H content of 3 ml of starting solutions A or C prior to any pH adjustments or extraction.

[c] The sensitivity of these experiments permits the detection of $\geq 0.5\%$ ^{3}H.

extraction of CI-581-^{3}H with chloroform were presumably due to the removal of labile tritium. The experimental details of the stability tests are described in Appendix A, and the results are summarized in Table 2.

The results may be summarized as follows : after extraction of CI-581-^{3}H with chloroform, the HCl ($\leqslant$1.0 *N*) aqueous phases contained $\leqslant$2% ^{3}H, whereas the 0.1 *N* NaOH aqueous phases contained about 42% ^{3}H (1 hr exposure) and 69% ^{3}H (18 hr exposure); the 1.0 *N* NaOH aqueous phases contained about 69% ^{3}H (1 hr exposure) and 77% ^{3}H (18 hr exposure). These observations indicate that the tritium in CI-581-^{3}H·HCl was relatively stable in aqueous HCl of concentrations $\leqslant$1.0 *N*, whereas as much as 77% of the tritium was labile in aqueous NaOH.

A chromatography study (TLC) of an aqueous phase containing gross quantities of tritium after extraction of CI-581-^{3}H with chloroform (Experiment 4*b*) detected $<$3% of the total tritium spotted; no tritium having the same R_f as authentic CI-581 was detected. These data suggest that the labile tritium remaining in the aqueous phase was ^{3}HHO; ^{3}HHO would evaporate from the thin layer plate and would not be detected.

Site of tritium label on CI-581-^{3}H·HCl.

Exchange of tritium from CI-581-^{3}H in aqueous NaOH suggested that the major portion of the label was alpha to the carbonyl group and that loss of tritium occurred through enolization. To explore this possibility, CI-581-^{3}H·HCl was reduced to the cyclohexanol analogue (III-^{3}H), thus blocking this potential route of tritium exchange in aqueous NaOH. The reduction of CI-581-^{3}H·HCl to the cyclohexanol derivative (III-^{3}H) was accomplished as follows : CI-581-^{3}H·HCl (151 mg) was mixed with 251 mg non-radioactive CI-581·HCl and converted (method described in footnote ***) to CI-581-^{3}H (free base), m.p. 91-91.5° C, having a measured specific activity of 3.6 mc/mmole. This product (300 mg) together with 25 ml diethyl ether was added to a stirred mixture of 240 mg $LiAlH_4$ and 280 mg $AlCl_3$ in 25 ml diethyl ether. The reaction mixture was stirred at room temperature for 1/2 hour before adding with stirring 0.25 ml H_2O, then 0.19 ml 25% NaOH, and finally 0.9 ml H_2O. The resulting precipitate was filtered and washed with diethyl ether. The ethereal filtrate containing the product was dried with 2 g $MgSO_4$ (anhydrous) and filtered. To the dry ethereal filtrate was added 0.5 ml isopropyl alcohol saturated with HCl in an attempt to obtain a crystalline hydrochloride salt of the product. A white oil formed. The ether was removed by evaporation, and the residue (III-^{3}H) was first dried at 40° C under vacuum and then dissolved in 0.1 *N* HCl. The specific activity of III-^{3}H was 3.7 mc/mmole which is in good agreement with that of the starting ketone (3.6 mc/mmole) and indicates that the tritium label remained intact during the reduction. The characterization data for III-^{3}H are summarized in Table 3.

TABLE 3. Summary of characterization of III-^{3}H.

Infrared analysis.

III-^{3}H had the same spectral pattern as authentic III; no carbonyl group was detected; some impurity was detected.

Ultraviolet analysis.

An extensive ultraviolet analysis study revealed that III-^{3}H had the same spectral pattern as authentic III; no CI-581 was detected; an unidentified impurity of 6-10% was detected; the concentration was 4.6 mg III-^{3}H (free base)/ml; total calculated yield = 230 mg.

Specific activity.

The total yield of III-^{3}H was 3.563 mc^{3}H; based on the quantitative ultraviolet analysis of III-^{3}H, the calculated specific activity = 3.7 mc/mmole; this specific activity is in good agreement with that of the starting ketone (3.6 mc/mmole) and indicates that the tritium label remained intact during the reduction.

Chromatography.

A TLC (Silica Gel G_F; Et_2O : MeOH : H_2O, 4 : 4 : 1) study on III-^{3}H revealed an unidentified radiochemical impurity of 8.2% in addition to two other components containing approximately equal amounts of tritium, one having the same R_f as authentic III and one having the same R_f as authentic CI-581·HCl.

Discussion. Since no CI-581 was detected in III-^{3}H using infrared and ultraviolet analytical techniques and because a large quantity (approximately 45%) of the tritium in the product behaved chromatographically the same as CI-581·HCl, one could conclude that CI-581-^{3}H was present in III-^{3}H as a trace chemical impurity of high specific radioactivity. However, because CI-581-^{3}H is known to exchange as much as 77% of the tritium label into aqueous NaOH and because essentially no exchange of tritium into aqueous NaOH from III-^{3}H was detected (see Table 4), it was concluded that no CI-581-^{3}H was present in III-^{3}H. The 1 : 1 distribution of tritium on the two ^{3}H spots of the thin layer plate was probably due to the separation of the *cis* and *trans* forms of III-^{3}H, the *cis* form behaving chromatographically more like a ketone due to hydrogen bonding with the nitrogen of the methylamino group.

Tritium label stability tests on III-^{3}H were conducted as follows : III-^{3}H was exposed to aqueous 0.1 *N* NaOH and 1.0 *N* NaOH for different periods of time and then extracted into chloroform. The total tritium content of each phase was determined. The details of the experiments are described in Appendix B, and the results are summarized in Table 4. The results indicate that no significant amount of tritium exchanged from III-^{3}H into aqueous NaOH. Since III-^{3}H was derived from CI-581-^{3}H and because as much as 77% of the tritium in CI-581-^{3}H was labile in aqueous NaOH while no detectable loss of tritium occurred from III-^{3}H in aqueous NaOH, it is reasonable to conclude that at least 77% of the tritium in CI-581-^{3}H·HCl is alpha to the carbonyl group.

TABLE 4. Results of tritium label stability experiments on III-^{3}H.

Experiment	Exchange conditions [a]		% ^{3}H in [b]	
	Medium	Time	Chloroform phase	Aqueous phase [c]
10	0.1 *N* NaOH	1 hr	97.2	0.3
11	1.0 *N* NaOH	1 hr	97.7	0.4
12	0.1 *N* NaOH	18 hr	97.8	0.5
13	1.0 *N* NaOH	18 hr	96.3	0.4

[a] All of the experiments were conducted at room temperature (approximately 25° C). See Appendix B.

[b] The % ^{3}H in the final separated phases is based on the measured ^{3}H content of 3 ml of starting solution E prior to any pH adjustments or extraction.

[c] The sensitivity of these experiments permits the detection of $\geqslant 0.3\%$ ^{3}H.

DISCUSSION.

Many aromatic compounds can be labelled with tritium by exchange in tritiated acids such as CF_3COO^3H [3]. In addition, the trifluoroacetic acid catalyzed tritium exchange reactions with alkyl benzenes have been shown to be specific for the isotopic hydrogen labelling of the aromatic nucleus [3-6]. Some compounds containing carbonyl groups can also be tritium labelled by exchange reactions. The alpha hydrogens of ketones are readily exchangeable in aqueous base [7]. Ketones that have been exchange-labelled with deuterium in acid media include acetone (in H_2SO_4, 94% exchange in three treatments) [7], cyclopentanone (exchange of alpha hydrogens in H_2SO_4, 102.9%; in H_3PO_4, 104.9%) [7], and cyclohexanone (exchange of alpha hydrogens in H_2SO_4, 82.6%; in H_3PO_4, 100.0%) [8]. Aliprandi and Cacace [8] have described the tritium labelling of acetophenone by exchange in an acid medium containing ^3H_2O and found that 98.3% of the total incorporated tritium was in the methyl group while 1.7% was in the aromatic nucleus.

From these observations, it is clear that aromatic hydrogens as well as the alpha hydrogens of ketones can be exchanged with isotopic hydrogen in acid media. In the present work it has been shown that, under the acid exchange conditions of CF_3COOH, ^3H_2O and Pt, at least 77% of the tritium in CI-581-^{3}H appears to be oriented alpha to the carbonyl group in the cyclohexanone ring. The question of whether the remaining 23% tritium is in the cyclohexanone ring or in the aromatic ring has not been resolved. Nevertheless, this study serves as another interesting example of how an organic compound containing aromatic hydrogens as well as alpha hydrogens of a

ketone can compete intramolecularly for tritium under acid exchange conditions.

ACKNOWLEDGEMENT.

We gratefully acknowledge Dr. R. F. Parcell for his helpful advice concerning the chemical properties of CI-581, and concerning the synthesis and stereochemistry of III-^{3}H; Mr. W. M. Pearlman for his advice and assistance in the preparation of the platinum catalyst used in the preliminary exchange reactions; Dr. J. M. Vandenbelt and his group for the ultraviolet and infrared analyses; Mr. C. E. Childs and his group for the elemental analyses; Dr. A. J. Glazko for his continued interest and support.

REFERENCES

1. MCCARTHY, D. A., CHEN, G., KAUMP, D. H. and ENSOR, C. — *J. New Drugs*, **5** : 21 (1965).
2. CHANG, T., DILL, W. A. and GLAZKO, A. J. — *Fed. Proc.*, **24** : 268 (1965).
3. ALIPRANDI, B. and CACACE, F. — *Annali di Chimica* (Rome), **51** : 397 (1961).
4. LAUER, W. M., MATSON, G. W. and STEDMAN, G. — *J. Amer. Chem. Soc.*, **80** : 6433 (1958).
5. LAUER, W. M., MATSON, G. W. and STEDMAN, G. — *Ibid.*, **80** : 6437 (1958).
6. LAUER, W. M. and STEDMAN, G. — *Ibid.*, **80** : 6439 (1958).
7. MURRAY, A., III and WILLIAMS, D. L. — *Organic Synthesis with Isotopes*, Part II, Interscience Publishers, Inc., New York, 1958, p. 1604.
8. *Ibid.*, p. 1605.
9. ALIPRANDI, B. and CACACE, F. — *Annali di Chimica* (Rome), **50** : 931 (1960).

APPENDIX A: TRITIUM STABILITY TESTS ON CI-581-^{3}H·HCl.

Stock solutions of CI-581-^{3}H·HCl in 0.01 *N* HCl (solution A) and in 0.2 *M* (pH 2) citric acid (solution C) having a concentration of about 20 μg/ml were prepared and analyzed for tritium content per 3 ml aliquots. In all of the following experiments, appropriate controls were employed using non-radioactive CI-581.HCl. *Experiment 1*, 3 ml aliquots of solutions A and C were allowed to stand 18 hours at room temperature. Solution A was adjusted to pH 9 with 0.1 *N* NaOH and solution C to pH 8 with 3.0 ml 0.5 *M* Na_3PO_4. Each solution was extracted with 6 ml chloroform, and the tritium content of each phase was determined. *Experiment 2*, Experiment 1 was repeated with this exception : solutions A and C were heated for one hour at 37° C rather than allowing them to stand at room temperature for 18 hours. *Experiment 3*, Experiment 1 was repeated with this exception : solutions A and C were allowed to stand at room temperature for one hour rather than for 18 hours. *Experiments 4a and 6*, A 3 ml aliquot of solution C was adjusted to 0.1 *N* NaOH with 2.35 ml 1.0 *N* NaOH and allowed to stand one hour at room temperature. It was then extracted with 6 ml chloroform, and the tritium content of each phase was determined. *Experiments 4b and 7*, A 3ml aliquot of solution C was adjusted to 1.0 *N* NaOH with 1.6 ml 4.0 *N* NaOH and allowed to stand one hour at room temperature. It was then extracted with 6 ml chloroform, and the tritium content of each phase was determined. *Experiment 5a*, A 3 ml aliquot of solution A was adjusted to 0.1 *N* HCl with 0.3 ml 1.0 *N* HCl and allowed to stand one hour at

room temperature. It was adjusted to pH 8-8.5 with 0.57 ml 0.5 *M* Na_3PO_4 and then extracted with 6 ml chloroform. The tritium content of each phase was determined. *Experiment 5*b, A 3 ml aliquot of solution A was adjusted to 1.0 *N* HCl with 1.0 ml 4.0 *N* HCl and allowed to stand one hour at room temperature. It was adjusted to pH 8-8.5 with 0.9 ml 4.0 *N* NaOH and 0.7 ml 0.5 *M* Na_3PO_4 and then extracted with 6 ml chloroform. The tritium content of each phase was determined. *Experiment 8*, Experiment 4*a* was repeated with this exception : the solution was allowed to stand 18 hours before extracting with chloroform. *Experiment 9*, Experiment 4*b* was repeated with this exception : the solution was allowed to stand 18 hours before extracting with chloroform. *Experiment 14*, Experiment 4*b* was repeated with this exception : the solution was allowed to stand 2 days before extracting with chloroform.

APPENDIX B: TRITIUM STABILITY TESTS ON III-^{3}H.

A stock solution of III-^{3}H in 0.01 *N* HCl (solution E) having a concentration of about 46 μg/ml was prepared and analyzed for tritium content per 3 ml aliquots. In all of the following experiments, a control using non-radioactive III was employed. *Experiment 10*, A 3 ml aliquot of solution E was adjusted to 0.1 *N* NaOH with 0.37 ml 1.0 *N* NaOH and allowed to stand one hour at room temperature. It was then extracted with 6 ml chloroform, and the tritium content of each phase was determined. *Experiment 11*, A 3 ml aliquot of solution E was adjusted to 1.0 *N* NaOH with 1 ml 4.0 *N* NaOH and allowed to stand one hour at room temperature. It was then extracted with 6 ml chloroform, and the tritium content of each phase was determined. *Experiment 12*, Experiment 10 was repeated with this exception : the solution was allowed to stand 18 hours before extracting with chloroform. *Experiment 13*, Experiment 11 was repeated with this exception : the solution was allowed to stand 18 hours before extracting with chloroform.