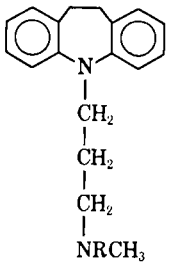
Functional Group Contribution in Ion-Pair Extraction of Tricyclic Amines

HO-LEUNG FUNG[▲] and YIM-HING OW

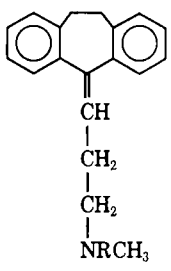
Abstract □ The use of ion-pair extraction data to obtain group contribution values necessitates an assumption of counterion independency. This assumption was examined in the present study, using the extraction of four tricyclic antidepressants with several anions as examples. It was found to be valid, and a consistent relative functional group value could be obtained when substitution on the tricyclic amines was distant from the charged center. However, when substitution was at the charged center, as exemplified by comparison of the methylated and demethylated antidepressants, the observed group contribution value was anion dependent.

Keyphrases □ Ion-pair extraction of tricyclic amine antidepressants—functional group contribution, linear free energy relationships □ Tricyclic amine antidepressants—ion-pair extraction, functional group contribution, linear free energy relationships □ Linear free energy relationships—tricyclic amine antidepressants □ Functional group contribution in ion-pair extraction—tricyclic amine antidepressants □ Counterion independency—interrelationship between ion-pair extraction and group contribution, tricyclic amine antidepressants

The functional group contribution approach, first proposed by Butler (1), has recently been applied to pharmaceutical systems to permit thermodynamic analyses of structure-activity relationships of drugs (2) and of buccal (3) and intestinal (4) absorption data of *n*-alkanoic acids. Individual functional group contribution values, *F* values, can be derived from various physicochemical measurements[1]. Recently, it has been suggested that these values can also be obtained from ion-pair extraction data (2). This method, however, necessarily assumes that *F* is independent of the counterion with which extraction is effected (see *Appendix*); otherwise, its usefulness would be severely limited. Validity of this assumption, on the other hand, not only would allow consistent *F* values to be obtained but it would also permit, in principle, *a priori* prediction of ion-pair extraction constants from previously documented group contribution values.



I: imipramine, R = CH_3
II: desipramine, R = H



III: amitriptyline, R = CH_3
IV: nortriptyline, R = H

[1] S. S. Davis, T. Higuchi, and J. H. Rytting, personal communication.

The present article is concerned with the preliminary testing of the validity and limitations of this assumption of counterion independency in the interrelationship between ion-pair extraction and group contribution. The ion-pair extractions of four tricyclic antidepressants (I–IV) and several anions were used as examples.

EXPERIMENTAL

Reagents—The tricyclic amines were donated by their respective manufacturers[2] and were used without purification. Inorganic counterions were present as their sodium or potassium salts, all of which were of analytical reagent grade. *p*-Toluenesulfonic acid was the monohydrate crystal. 2-Naphthalenesulfonic acid was recrystallized from an ether–chloroform mixture, and the white crystals obtained were dried in a vacuum oven for 8 hr.

A 0.1 *N* sulfuric acid solution was prepared from standardized 1 *N* solution[3] and distilled water. Chloroform (analytical grade) was washed with distilled water three or four times before it was cosaturated with 0.1 *N* sulfuric acid solution; these solvents were then used immediately.

The Extraction Constant—The extraction of ion-pairs into the organic layer may be represented by Eq. 1 (5):

$$BH^+_{aq} + X^-_{aq} \rightleftharpoons BH^+X^-_{org} \qquad \text{(Eq. 1)}$$

where BH^+_{aq} represents the protonated amine in the aqueous phase; X^-_{aq} is the anion in the aqueous phase, and $BH^+X^-_{org}$ is the ion-pair in the organic phase. The ion-pair extraction constant, *E*, is defined as in Eq. 2:

$$E = \frac{[BH^+X^-]_{org}}{[BH^+]_{aq}[X^-]_{aq}} \qquad \text{(Eq. 2)}$$

In the absence of side reactions, such as dimerization and tetramerization of the ion-pair in the aqueous or organic phase (6), the extraction constant can be directly obtained from the slope of plot of $[BH^+X^-]_{org}$ *versus* $[BH^+]_{aq}[X^-]_{aq}$. In the concentration ranges employed, all of the systems studied showed good linear plots. It was then possible to neglect contributions from side reactions. In some cases, a small positive intercept was obtained, presumably due to the contribution from the partitioning of the free base and/or the sulfate ion-pair. This, however, did not affect the magnitude of the extraction constant obtained from the slope of the plot.

Procedure for Determination of Ion-Pair Extraction Constants—The tricyclic amine hydrochloride and counterions were dissolved separately in 0.1 *N* sulfuric acid solution presaturated with chloroform. Different volumes of the two solutions were mixed and adjusted to volume so as to provide various initial concentrations of the amine and counterion ($[amine]_{total}$, 10^{-4}–10^{-3} *M*; $[X^-]_{total}$, 10^{-4}–10^{-1} *M* depending on X^-). The aqueous phase was then added to a known volume of chloroform, previously saturated with 0.1 *N* sulfuric acid, in a centrifuge tube. The solutions were tumbled end-over-end continuously at 25.0 ± 0.2° for at least 20 min. The phases were then allowed to separate on standing or centrifuging, and an aliquot of the aqueous phase was obtained through a pipet, the tip of which was wrapped with glass wool to remove any minute globules of chloroform. The concentration of unextracted amine

[2] Desipramine hydrochloride and imipramine hydrochloride from Geigy Pharmaceuticals, Division of Ciba-Geigy Corp., Ardsley, N. Y.; nortriptyline hydrochloride from Eli Lilly and Co., Indianapolis, Ind.; and amitriptyline hydrochloride from Merck Sharp and Dohme, West Point, Pa.

[3] Dilut-it, J. T. Baker Chemical Co., Phillipsburg, N. J.

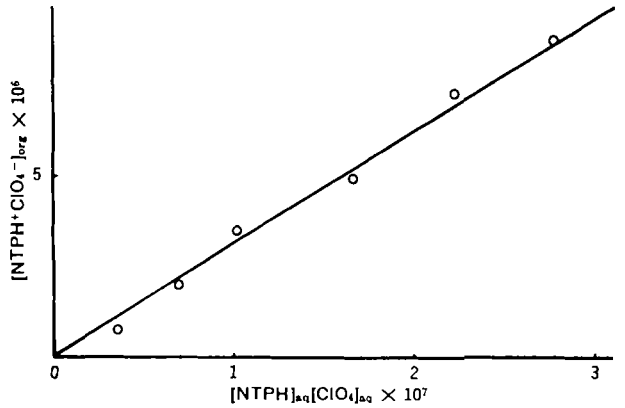


Figure 1—*Extraction of nortriptyline cation by perchlorate by single extraction procedure.*

was then determined spectrophotometrically at the appropriate wavelength. Since the pH was sufficiently low so that the concentration of the free base, $[B]_{aq}$, could be ignored, $[BH^+X^-]_{org}$ was obtained by $[amine]_{total} - [BH^+]_{aq}$ (with the appropriate volume correction factor). In cases where $[BH^+X^-]_{org}$ was experimentally determined by UV measurement of the organic phase, the experimental concentration was found to be the same as the calculated one. When $[X^-]_{total} \gg [amine]_{total}$, $[X^-]_{aq}$ was approximated by $[X^-]_{total}$; otherwise, appropriate correction was made to account for loss due to ion-pair formation.

When the anion interfered with the UV determination of the amine in the aqueous phase, a double-extraction procedure was employed in which the aqueous aliquot was made alkaline with 0.1 *N* NaOH and subsequently extracted with cyclohexane. The amine concentration was then determined by UV measurement of the cyclohexane phase, using a calibration curve obtained under identical conditions.

Strongly buffered solutions were not used in this study, because formation of ion-pairs between buffer salts and all the amine cations was extensive. The effect of ionic strength on the extraction constants was found to be small, since addition of sodium sulfate had a negligible effect on the extractions. Thus, under the experimental conditions described, reproducible results were obtained without a rigorous control of pH and ionic strength of the aqueous phase.

RESULTS AND DISCUSSION

Figure 1 shows a typical plot of Eq. 2. The data shown were for the single extraction of nortriptyline with perchlorate ($[amine]_{total} = 5.6 \times 10^{-5}$ *M*; $[ClO_4^-]_{total} = 6 \times 10^{-4}$–$6 \times 10^{-3}$ *M*). The slope obtained for this plot, which is identical to the extraction constant *E*, was found to be 31.2 M^{-1}. Figure 2 shows a plot of Eq. 2 for the same extraction system, except that a double-extraction procedure (see

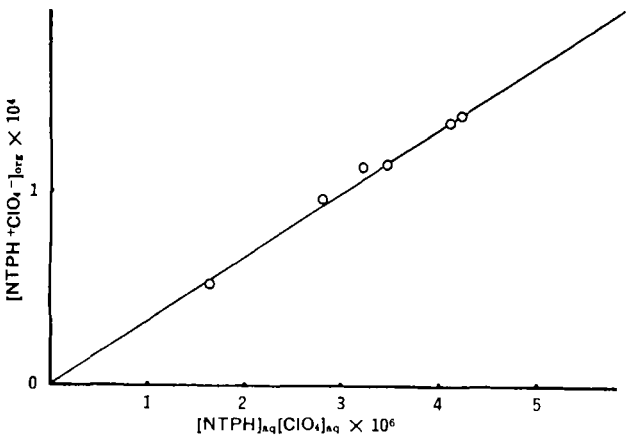


Figure 2—*Extraction of nortriptyline cation by perchlorate by double extraction procedure.*

Table I—Log *E* of Tricyclic Amines with Various Anions[a]

Anion	Log *E*			
	Imipramine	Desipramine	Amitriptyline	Nortriptyline
Chloride	2.14	0.42	2.30	0.49
Bromide	2.71	0.94	2.90	1.10
Chlorate	2.78	1.43	2.98	1.51
Nitrate	3.06	1.17	—	1.32
Iodide	3.61	2.31	—	—
p-Toluenesulfonate	—	—	3.74	3.01
2-Naphthalenesulfonate	—	—	4.28	3.98

[a] Solvent was chloroform.

Experimental section) was used and the concentrations of the extracting species were higher ($[amine]_{total} = 9 \times 10^{-4}$ *M*; $[ClO_4^-]_{total} = 2 \times 10^{-3}$–$6 \times 10^{-2}$ *M*). The extraction constant was found to be 32.6 M^{-1} from this plot. It can be seen that within the ranges of concentration used, the extraction constant appeared to be relatively insensitive to concentrations of the species added and, hence, to any slight variation of pH and ionic strength resulting from different initial concentrations of the amine hydrochloride and the counterion. Similar results were obtained for all extraction systems. Table I tabulates log *E* of the four tricyclic amines with the anions studied. These extraction constants may be examined in two separate fashions.

First, the azepine tricyclic compounds [imipramine (I) and desipramine (II)] may be compared with the cycloheptene series [amitriptyline (III) and nortriptyline (IV)]. Compounds I and III and Compounds II and IV, respectively, are structurally identical except for one constituent group connected with the tricyclic ring; *i.e.*, the >N—CH_2– group (group *a*) in I and II is replaced by the >C=CH– group (group *b*) in III and IV. Thus, it is possible to obtain the group contribution value of *a* relative to *b* from extraction constants of either the imipramine-amitriptyline pair or the desipramine-nortriptyline pair.

Figure 3 shows a linear free energy plot in which the logarithms of the ion-pair extraction constants of the azepine tricyclic amines were plotted against their corresponding cycloheptene tricyclic analogs. For the anions shown, a linear relationship was obtained. The slope of the line was, within experimental errors, unity and the *x*-intercept was found to be 0.15. The latter value, therefore, represented the group contribution value of the >C=CH— moiety relative to that of the >N—CH_2– moiety. It is apparent that in this instance, a consistent group value could be obtained irrespective of: (*a*) the choice of counterion, and (*b*) the extent of substitution at the terminal aliphatic nitrogen atom. The cycloheptene derivatives were 1.4 times more extractable than their corresponding azepine compounds.

Second, the tetriary amines (I and III) may be compared with their corresponding secondary amines (II and IV) to obtain the group contribution value of the *N*-substituted methyl moiety. A

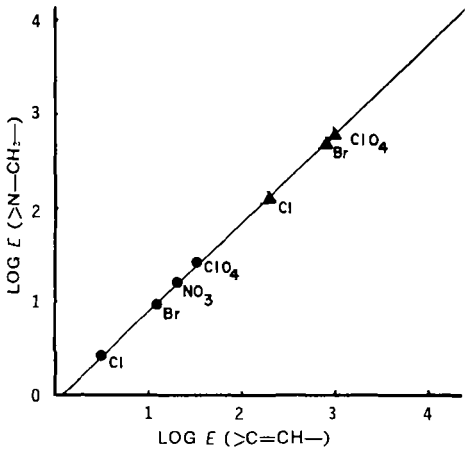


Figure 3—*Linear free energy plot of extraction constants: azepine* versus *cycloheptene tricyclic antidepressants. Key:* ▲, *imipramine* versus *amitriptyline; and* ●, *desipramine* versus *nortriptyline.*

Table II—Calculated Group Contribution Constant Values, Δ log E, for the N-Substituted Methyl Group from Different Ion-Pairs

Anion	log E_{I} − log E_{II}	log E_{III} − log E_{IV}
Nitrate	1.89	—
Bromide	1.77	1.80
Chloride	1.72	1.81
Chlorate	1.35	1.47
Iodide	1.30	—
p-Toluenesulfonate	—	0.73
2-Naphthalenesulfonate	—	0.30

similar linear free energy plot (Fig. 4) showed, however, that this value was highly anion dependent (Table II). For example, the extraction of the tertiary amines was approximately 80 times more favorable than that of the secondary amines with the nitrate anion; for the 2-naphthalenesulfonate anion, the tertiary amines were only about twice more extractable.

These preliminary data, therefore, indicated some serious limitations in the assumption of counterion independency in the utilization of ion-pair extraction constants to obtain group contribution values. When alteration of substituent groups were carried out away from the charged center, as when azepine derivatives were compared with the cycloheptene derivatives, the assumption seemed to hold and a consistent group contribution value could be obtained from ion-pair extraction data. Conversely, *a priori* prediction of ion-pair extraction constants might then be possible in these systems. However, the assumption was shown to be invalid when substitution was carried out at the charged head, in which case the mutual application of group contribution approach and ion-pair extraction was not appropriate.

The observed results may be rationalized in terms of the fundamental premise of the group contribution approach vis-à-vis the the energetics of ion-pair extraction. The group contribution approach is essentially an extrathermodynamic one, utilizing linear free energy relationships (see *Appendix*). Its validity is, therefore, based on the assumption that the total free energy change involved for the process is additively composed of *independent* contributions from the constituent groups[1]. The addition or alteration of a constituent group must not, according to this assumption, cause a concomitant change in the contributions of other functional groups. This criterion, when applied to ion-pair extraction, requires that the mechanism of electrostatic interaction and subsequent solvation of the ion-pair, the main driving force for extraction, be essentially unchanged on substitution; otherwise, the relative contributions from functional groups would be severely affected. This criterion was apparently met when the azepine tricyclic compounds were compared with their corresponding cycloheptene derivatives to obtain the group contribution value of the >C=CH— moiety relative to the >N—CH_2— group. This was expected since alteration of the constituent group was too distant from the charged center to affect significantly the mechanism of electrostatic interaction between the terminal nitrogen cation with each individual anion and subsequent solvation of the ion-pair. On the other hand, N-methyl substitution at the charged head of the tricyclic amines probably affects the hydrogen-bonding capability and/or the steric arrangement of the cation so that the mechanism of electrostatic interaction and solvation becomes anion dependent.

APPENDIX

If the group contribution approach can be applied to ion-pair extraction systems, it will then be possible to write a linear free energy relationship (Eq. 1) to describe the extraction:

$$\Delta G_{A(X)} = \sum_{Ai} \Delta G_{Ai(X)} \qquad \text{(Eq. A1)}$$

where $\Delta G_{A(X)}$ is the "overall" free energy change involved for the extraction of cation A and anion X; A_i represents the ith functional group which constitutes A; and $\Delta G_{Ai(X)}$, therefore, represents the partial free energy contribution for the extraction of the functional group, A_i, with anion X. Assuming that activity coefficient corrections are negligible:

$$\Delta G_{A(X)} = -RT \ln E_{A(X)} \qquad \text{(Eq. A2)}$$

where $E_{A(X)}$ is the ion-pair extraction constant for ions A and X. From Eqs. A1 and A2:

$$\log E_{A(X)} = \sum_{Ai} \log \epsilon_{Ai(X)} \qquad \text{(Eq. A3)}$$

where $\epsilon_{Ai(X)}$ represents the partial contribution of functional group A_i to the total ion-pair extraction. This parameter is similar to the F value as defined by Higuchi and Davis (2).

A relationship similar to Eq. A3 can be written for any other cation, *e.g.*, B; thus:

$$\log E_{B(X)} = \sum_{Bi} \log \epsilon_{Bi(X)} \qquad \text{(Eq. A4)}$$

If the functional groups that constitute both A and B are all identical except for one functional group, *e.g.*, Group a in Compound A is replaced by Group b in Compound B, it follows that:

$$\log E_{A(X)} - \log E_{B(X)} = \log \epsilon_{a(X)} - \log \epsilon_{b(X)} \qquad \text{(Eq. A5)}$$

This relationship is also valid for any other counterion, *e.g.*, Y:

$$\log E_{A(Y)} - \log E_{B(Y)} = \log \epsilon_{a(Y)} - \log \epsilon_{b(Y)} \qquad \text{(Eq. A6)}$$

The terms on the right-hand side of Eqs. A5 and A6 then represent the group contribution value of Group a relative to Group b as obtained from the ion-pair extraction with anions X and Y, respectively. These two values, as well as those obtained with other anions, should be identical if ion-pair extraction data are to be used to determine functional group contribution values. It follows then that the relative extraction constant of cation A over cation B is also anion independent:

$$\log E_A - \log E_B = \text{constant for all anions} \qquad \text{(Eq. A7)}$$

Equation A7 is, in effect, a Hammett $\sigma\rho$ relationship (7) extended to ion-pair extraction. A similar equation can, of course, be derived when substitution in the anion is considered instead.

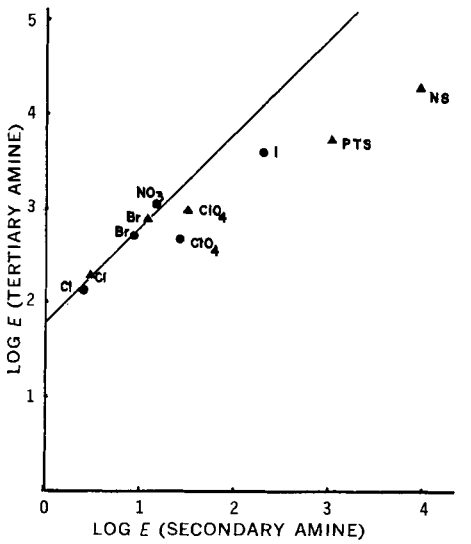


Figure 4—*Linear free energy plot of extraction constants: methylated* versus *demethylated tricyclic antidepressants. Key:* ●, *imipramine* versus *desipramine; and* ▲, *amitriptyline* versus *nortriptylene. PTS* = p-*toluenesulfonate, and NS* = *2-naphthalenesulfonate.*

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Influence of Gibberellic Acid on Growth, Flowering, and Alkaloidal Content of *Atropa belladonna* L. Grown in Egypt

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Abstract □ Gibberellic acid seed treatment and dripping at the fourth to fifth leaf stage of *Atropa belladonna* L. increased the stem length, number of leaves, and number of branches. Gibberellic acid had a differential effect on the area of the leaf at different internodes. The dry weight of shoots of treated plants was increased, especially at the full-flowering stage. A pronounced increase in the alkaloidal content was obtained in the shoots of treated plants at 50 mg./l. gibberellic acid, especially at flowering phases.

Keyphrases □ Gibberellic acid—effect on growth, flowering, and alkaloidal content of *Atropa belladonna* L. □ *Atropa belladonna* L.—effect of gibberellic acid on growth, flowering, and alkaloidal content □ Plant growth regulators—effect of gibberellic acid on *Atropa belladonna* L.

Gibberellic acid has been reported to influence the vegetative growth, flowering process, and major metabolic pathways in plants (1–5). In addition, its influence on the biosynthesis of some active constituents of medicinal plants has been studied, especially on *Datura stramonium* L., *Catharanthus roseus* L., *Hyoscyamus niger* L., and *Atropa belladonna* L. (6–12).

This work was carried out to study the effect of soaking the seeds in gibberellic acid and then dripping gibberellic acid on the same plants raised from the treated seeds on the growth, development, and alkaloidal pattern of *A. belladonna* L. at different physiological stages.

EXPERIMENTAL

Growing the Plant—Seeds of *A. belladonna* L.[1] were germinated in culture flats. After 1 month, the uniform young seedlings (two to three leaves) were transplanted into small pots and were kept there for an additional month. The plants were then transferred to larger pots (30 cm. in diameter), containing 12 kg. of soil[2], until the end of the experiment. At 1-month intervals, plants within each pot were fertilized with 3 g. of a mixture containing calcium superphosphate, potassium sulfate, and calcium nitrate.

Treatment—The seeds were soaked for 24 hr. in different concentrations of a freshly prepared aqueous solution of gibberellic acid[3] (25, 50, and 100 mg./l.). Furthermore, 2 ml. of the same concentrations of gibberellic acid was dripped on the terminal buds and the young unfolded leaves of the plants raised from the treated seeds. Dripping of gibberellic acid was carried out, using a graduated pipet, at the fifth to sixth leaf stage. Dripping was repeated four times at 4-day intervals.

Samples of the shoots and roots from each treatment were drawn at random for dry weight determination before flowering, at flower budding, at full flowering, and at fruiting stages. The samples were dried to constant weights in a circulating hot air oven at 70°, reduced to powder (40 mesh), and stored in airtight containers for chemical analysis. The leaf area per plant (after 10 days from the end of gibberellic acid dripping) was determined using a planimeter.

Growth and Flowering Studies—The length of the main stem and each of the successive 10 internodes, as well as the number of leaves and branches, was recorded at 10-day intervals. Flowering and fruiting dates were also recorded. The flowering date was calculated as the days from germination to the appearance of the first visible flowerbud for each treatment. The numbers of flowers and fruits were recorded daily. In all cases, the mean of 10 plants for each treatment was calculated.

Alkaloidal Content Determination—Extraction of the alkaloids from the plant samples was carried out according to the procedure of Allport and Wilson (13). The spectrophotometric determination of the alkaloids was performed using the method of Durick *et al.* (14). The total alkaloidal content was calculated as milligrams hyoscyamine per gram dry weight as well as per dry weight of every plant organ.

Statistical Analysis—Available data were subjected to analysis of variance to calculate the *F* test and the least significant difference at 0.01 according to the design of complete randomized plots with interaction (15). The least significant difference was calculated between

[1] The seeds of *A. belladonna* L. were supplied through the courtesy of Prof. Dr. R. T. Voigt, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60680.

[2] Loamy clay soil having the following mechanical analysis: sand, 24%; silt, 47%; and clay, 29%.

[3] Gibberellic acid was supplied by Merck Sharp & Dohme, Rahway, N. J.