alcoholysis, should not be entirely disregarded. Additional experiments are needed to obtain a satisfactory picture of the reaction. Further work, aimed at collecting kinetic data for the dissociation of I and of other pharmacologically active sulfonylureas in different media, is now in progress.

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Molecular Association of Barbital and Caffeine in 2:1 Crystalline Complex

B. M. CRAVEN and G. L. GARTLAND

Abstract ☐ The crystal structure of the 2:1 complex of barbital with caffeine has been determined by X-ray diffraction methods. The crystals are triclinic, space group $P\bar{1}$, with $a = 14.627$, $b = 14.160$, $c = 6.902$ Å, $\alpha = 95°15'$, $\beta = 92°48'$, and $\gamma = 100°45'$, and with four barbital and two caffeine molecules in the cell. The block-diagonal least-squares refinement of 496 atomic positional and thermal parameters, based on 4665 X-ray intensity data, gave a final R factor of 0.05. The structure consists of ribbons of barbital molecules linked by NH···O=C hydrogen bonds. Caffeine molecules are bound to the ribbon by an NH···N(9) hydrogen bond and by an unusual interaction involving C(8)H with two barbital oxygen atoms. Weak interactions of nonhydrogen-bonded caffeine carbonyl groups with barbital carbonyl groups may also be important in this crystal. There is minimal overlap of the flat ring systems of the component molecules.

Keyphrases ☐ Molecular association—barbital–caffeine 2:1 crystalline complex ☐ Barbital, molecular association—caffeine in 2:1 crystalline complex ☐ X-ray diffraction—barbital–caffeine 2:1 crystalline structure, determination

Barbiturates (1–3) and xanthines (4, 5) form crystalline complexes with a variety of other molecular species as well as with each other (6–8). The crystal complex of barbital with caffeine was chosen for study because consideration of the structure of the component molecules (Fig. 1) shows that the nature of barbital–caffeine association must differ in two important respects from that found in previously determined crystal structures of complexes of purines and pyrimidines.

The strong association by pairs of hydrogen bonds, which occurs in complexes of barbiturates with adenine derivatives (3, 9) and which is analogous to the hydrogen bonding in the crystal structures of nucleic acid base pairing model systems (10), cannot occur between barbital and caffeine. As a hydrogen-bonding donor caffeine can at most form a weak C(8)H hydrogen bond

The stacking together of flat molecules with extensive overlap of their π-bonded ring systems has been found in complexes of tetramethyluric acid with pyrene (11) and caffeine with 5-chlorosalicylic acid (5). This type of interaction, which has been termed polarization bonding, is postulated as an important cohesive factor in xanthine complexes (12). However, in the complex of caffeine with barbital, overlap of the two flat ring systems is largely prevented by the ethyl groups, which shield each side of the barbital ring.

The crystal structure of the 2:1 barbital–caffeine complex was determined to reveal the detailed geometry of molecular association.

EXPERIMENTAL

Triclinic crystals (m.p. 142°) of the complex were obtained as described by Higuchi and Lach (6). The lattice parameters are $a = 14.627$, $b = 14.160$, $c = 6.902$ Å, $\alpha = 95°15'$, $\beta = 92°48'$, and $\gamma = 100°45'$. The space group is $P\bar{1}$, and there are four barbital and two caffeine molecules in the unit cell. The X-ray intensity data (4665 reflections) were collected on a four-circle automatic diffractometer using CuKα radiation. All 40 nonhydrogen atoms of the crystal chemical unit were found in the first E map, derived from an application of the direct method of phase determination similar to that described by Karle (13). All 34 hydrogen atoms were subsequently found in a difference Fourier synthesis. The positional and anisotropic thermal parameters for heavier atoms and positional and isotropic thermal parameters for hydrogen atoms were refined by a block-diagonal least-squares procedure to give a final R factor of 0.05.

Description of the Structure—The crystal structure consists of stacks of hydrogen-bonded ribbons, one of which is shown in Fig. 2. The backbone of the ribbon is made up of the barbital molecules,

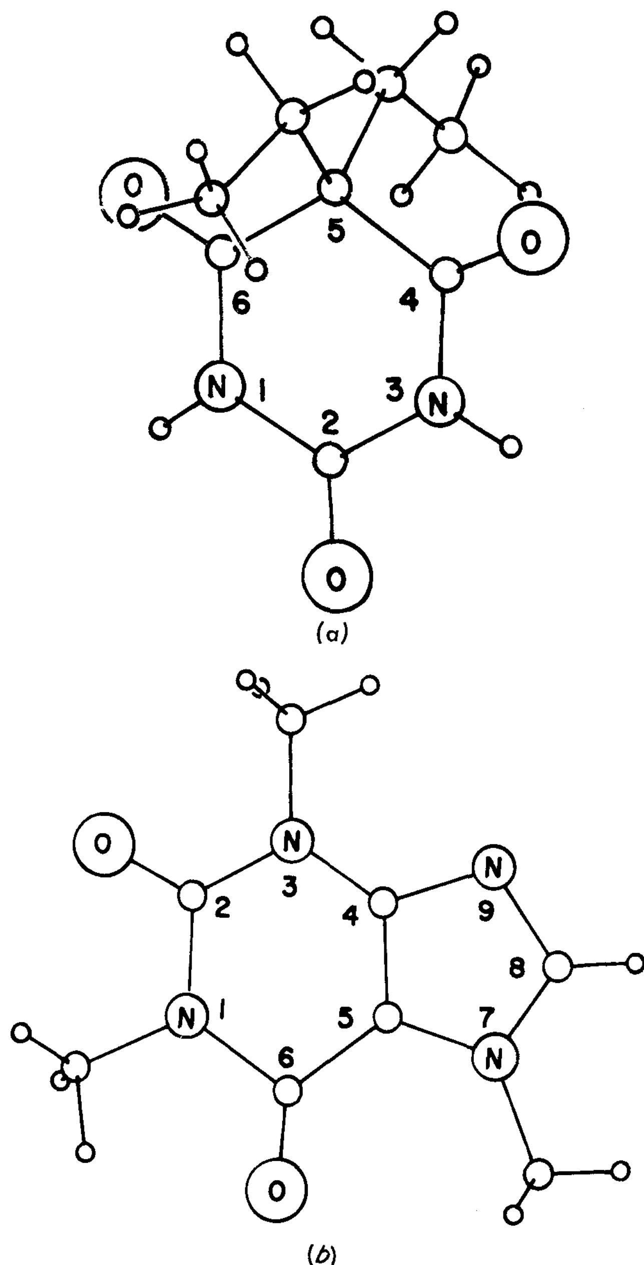

Figure 1—*Molecular structure and atomic nomenclature for* (a) *barbital and* (b) *caffeine. Unlabeled atoms are carbon and hydrogen, shown as large and small spheres, respectively. Both molecules are drawn with the stereochemistry including hydrogen atom orientation, which was determined in the crystal structure determination of the 2:1 complex. The barbital molecule is Molecule B viewed as in Fig. 2.*

which are linked by NH···O=C hydrogen bonds. The ribbons are puckered so as to avoid close contact between the nonhydrogen-bonded carbonyl oxygen atom O(6) of barbital Molecule A and the ethyl group of Molecule B′ further along the same ribbon. Each caffeine molecule is associated with a ribbon by accepting an NH···N(9) hydrogen bond from barbital and by an unusual type of interaction C(8)H···O(2), with two barbital carbonyl groups. The angles C—H···O are 144° and 130° with e.s.d.'s of 4°, and the corresponding H···O distances are 2.32 and 2.64 Å with e.s.d.'s of 0.05 Å. Only the former distance is appreciably shorter than the sum of the appropriate van der Waals' radii (2.6 Å) as listed by Pauling (14).

Figure 2 shows that the ethyl and methyl groups of the barbital and caffeine molecules generally project outward from the border of the hydrogen-bonded ribbon. The mode of assembly of ribbons to form the three-dimensional crystal structure gives rise to close packing of these alkyl groups, as well as partial overlap of stacked flat ring systems.

There are 11 H···H distances less than 2.6 Å between alkyl hydrogen atoms of adjacent ribbons, of which the shortest is 2.32 Å. In stacking along the z-direction, *i.e.*, above or below the page in Figs. 2 and 3, there are five such distances between barbital ethyl groups, four between barbital ethyl and caffeine methyl groups, and one between caffeine methyl groups. There are two such distances between barbital ethyl groups of laterally adjacent ribbons.

The superposition of the flat ring systems of caffeine and barbital in the crystal structure is shown in Fig. 3. Corresponding interatomic distances are listed in Table I.

Details concerning molecular structure and stereochemistry in this crystal structure will be reported.

DISCUSSION

In barbiturate crystal structures, there are two types of intermolecular hydrogen-bonding linkage. These may be termed "cyclic" if the molecules are linked by a pair of NH···O=C hydrogen bonds, as between barbital A::::A′ and barbital B::::B′ (Fig. 2), or "noncyclic" if only one hydrogen bond is formed, as between barbital A····barbital B. Although there are both cyclic and noncyclic barbital linkages in the 2:1 complex with caffeine and also in barbital polymorph I (15), there are crystal structures in which the hydrogen bonding is exclusively cyclic, as in barbital polymorph II (15) and amobarbital polymorphs I and II (16), or exclusively noncyclic, as in γ-methylamobarbital (17).

Further possibilities for hydrogen-bonding variations arise when there are insufficient NH or other donor groups to form crystal structures in which all three barbiturate carbonyl oxygen atoms are hydrogen bonded. Under these circumstances, C(2)—O(2) and C(4)—O(4)[1] usually accept one hydrogen bond each and C(6)—O(6) is not hydrogen bonded as in the 2:1 complex of barbital with caffeine (Fig. 2), the 1:2 complex of phenobarbital with 8-bromo-9-ethyladenine (3), barbital I, γ-methylamobarbital, and amobarbital I and II. In barbital II, both C(4)—O(4) and C(6)—O(6) are hydrogen bonded and C(2)—O(2) is not. These crystal structures all contain hydrogen-bonded ribbons with different geometries. In vinbarbital I (18), heptabarbital (19), and barbital IV (20), C(4)—O(4) forms two hydrogen bonds while C(6)—O(6) and C(2)—O(2) are not hydrogen bonded. There is no case as yet in which only C(2)—O(2) is hydrogen bonded.

Thus the barbital–barbital hydrogen bonding in the 2:1 complex with caffeine represents only one of the variety of different modes that have been found in barbiturate crystal structures.

Although only three crystal structures containing caffeine have so far been determined, the hydrogen-bonding behavior of this molecule is more consistent. In caffeine hydrate (21), the 1:1 complex of caffeine with 5-chlorosalicylic acid (5) and in the presently reported structure (Fig. 2), atom N(9) is the acceptor for a normal hydrogen bond [HOH···N(9), —COOH···N(9), and >NH···N(9), respectively], while the caffeine carbonyl groups C(2)—O(2) and C(6)—O(6) are nonhydrogen bonded, or at most these oxygen atoms are acceptors in very weak CH···O interactions.

While such consistency may be fortuitous, it suggests that for caffeine the N(9) hydrogen-bonding acceptor site is favored over the carbonyl oxygen atoms.

Crystal structures containing caffeine are also similar in that the C(8)H bond of the caffeine is directed more or less toward a carbonyl oxygen atom of another molecule. The occurrence and geometry of CH···O hydrogen bonds in xanthine and other crystal structures have been reviewed by Sutor (22). More recently, Donohue (23) has doubted the inference that H···O distances between 2.2 and 2.6 Å in CH···O *interactions* are *hydrogen bonds*, at least in the sense that this term is used for O—H···O, N—H···O, and N—H···N systems. Whether or not these are called hydrogen bonds, the recurrence of H···O distances less than 2.5 Å between caffeine C(8)H and carbonyl oxygen atoms suggests that an attractive interaction is present which is stronger than the usual van der Waals' effect.

[1] In the isolated molecule (Fig. 1), C(4)—O(4) and C(6)—O(6) are symmetry-related but distinct from C(2)—O(2).

Table I—Intermolecular Distances[a]

Hydrogen Bonding Distances[b]		Other Intermolecular Distances Involving Caffeine[c]			
N(1)A···O(2)B	2.98 Å	N(1)C···O(2)B	3.40 Å	C(2)C···O(2)A	3.04 Å
N(3)A···O(4)A	2.94	N(3)C···C(8)C	3.35	O(2)C···C(2)A	3.36
N(1)B···N(9)C	2.99	C(4)C···C(8)C	3.31	O(2)C···N(3)A	3.41
N(3)B···O(4)B	2.93	C(4)C···N(9)C	3.28	N(3)C···O(2)A	3.21
		C(5)C···N(9)C	3.42	C(3)C···O(4)A	3.34
		C(6)C···N(1)B	3.53	C(5)C···C(8)C	3.44
		C(8)C···N(3)C	3.35	C(6)C···O(2)B	3.40
C(8)H···O(2) distances		N(9)C···C(4)C	3.28	O(6)C···C(2)B	3.02
		N(9)C···N(9)C	3.39	O(6)C···O(2)B	3.22
C(8)C···O(2)A	3.16 Å			O(6)C···N(3)B	3.15
C(8)C···O(2)B	3.35			N(7)C···C(8)C	3.41
				N(7)C···N(9)C	3.40
				C(7)C···N(9)C	3.43
				C(8)C···N(7)C	3.41
				C(8)C···C(5)C	3.44
				N(9)C···N(7)C	3.40
				N(9)C···C(7)C	3.43

[a] Atomic nomenclature is as in Fig. 1. The labeling A, B, or C refers to barbital molecules (A and B as shown in Fig. 2) and caffeine (C). The barbital ring systems for both Molecules A and B are numbered so that C(6)—O(6) is the nonhydrogen-bonded carbonyl group. E.s.d.'s in the interatomic distances range between 0.005 and 0.009 Å. [b] See Fig. 2 for the distances involving hydrogen-bonded hydrogen atoms. [c] For each distance, the atom listed on the left belongs to the caffeine molecule shown centrally in either Fig. 3*a* or 3*b*. The column at the left gives distances to molecules "above," *i.e.*, in the positive *z*-direction, as in Fig. 3*a*; the column at the right gives distances to molecules "below," *i.e.*, in the negative *z*-direction, as in Fig. 3*b*.

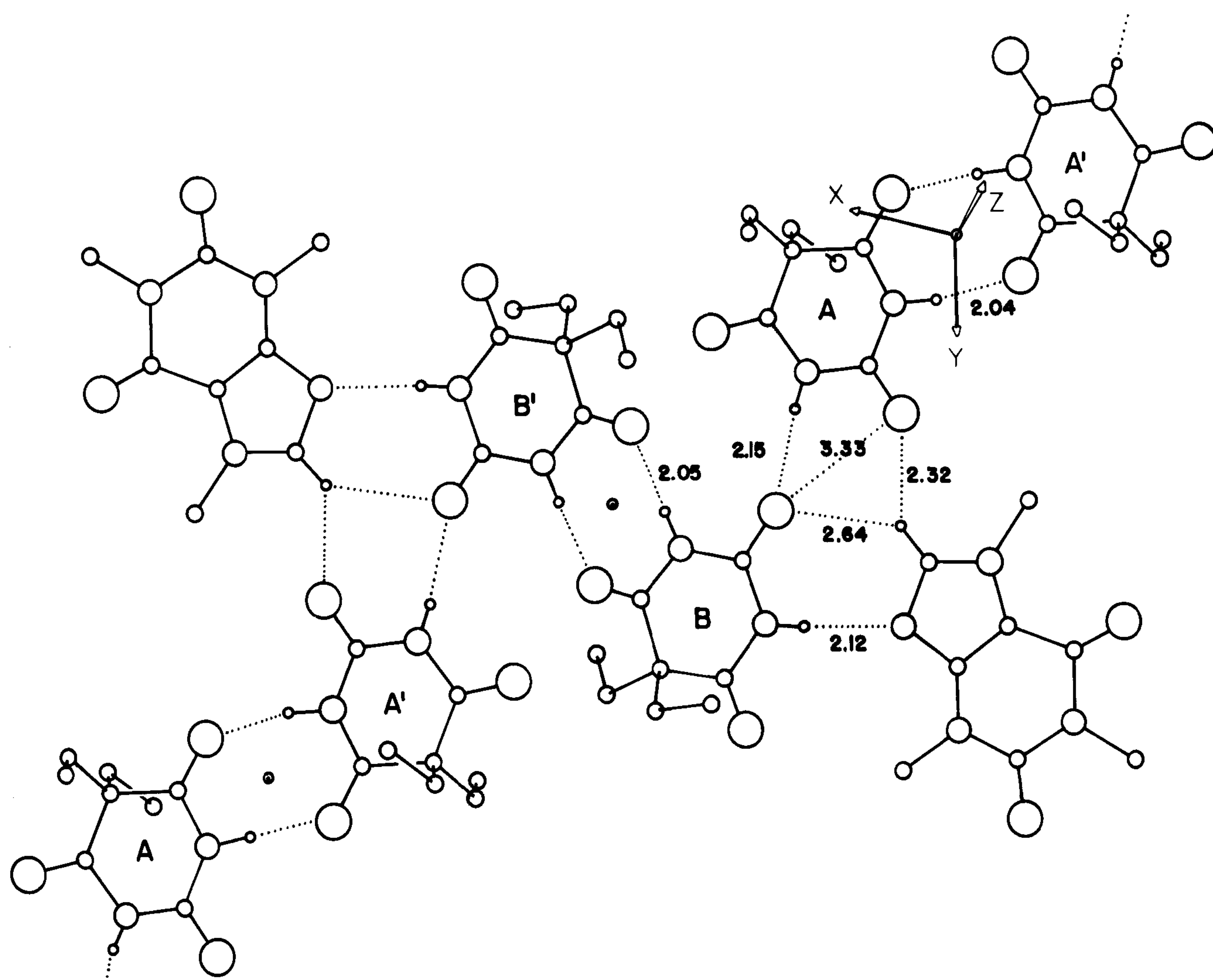

Figure 2—*The hydrogen-bonded ribbon structure observed in the crystal structure of the 2:1 complex of barbital and caffeine. The ribbon repeating unit is from barbital A (bottom left) to barbital A' (upper right). Barbital Molecules A and B are crystallographically independent. The ribbons are viewed normal to the plane of the caffeine five-membered ring. Spheres of decreasing size represent oxygen, nitrogen, carbon, and hydrogen-bonded hydrogen atoms, respectively.*

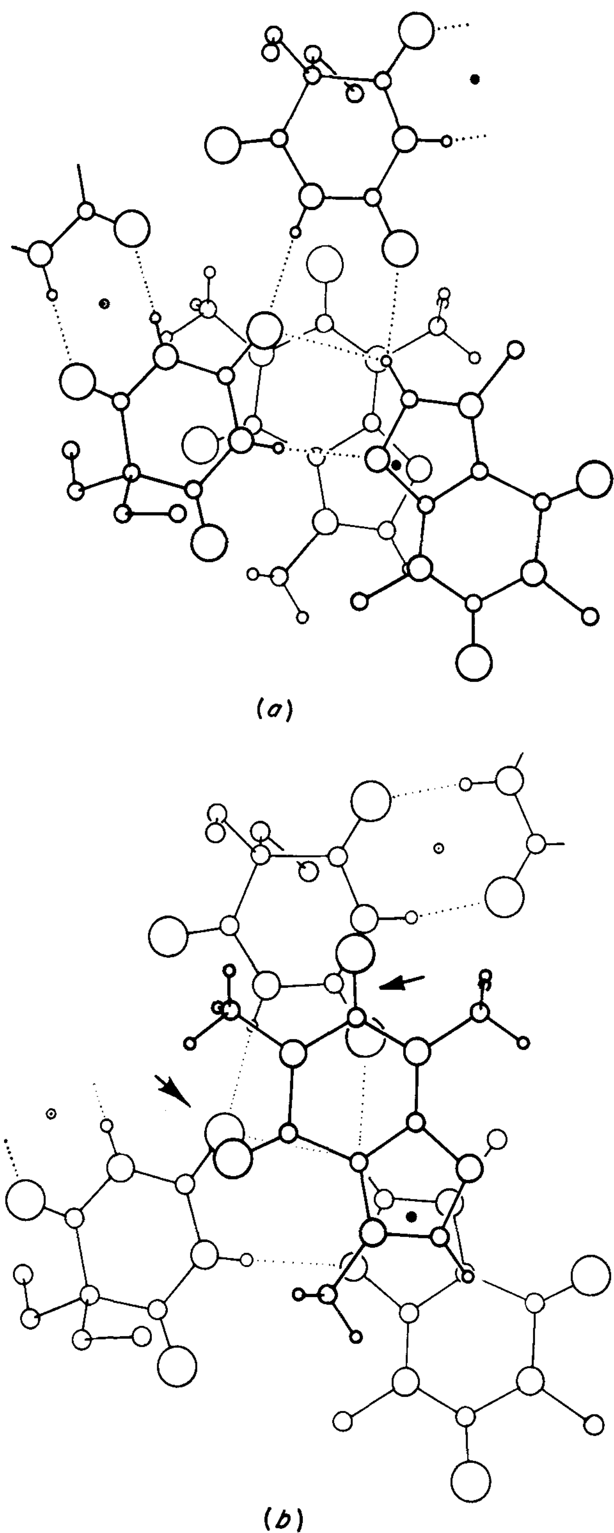

Figure 3—*Molecular overlap in the crystal structure of the 2:1 complex of barbital with caffeine. A central caffeine molecule is shown overlapped by the hydrogen-bonded ribbon "above" in* (a) *and "below" in* (b), *where "above" corresponds to the positive z-direction in the crystal. The view is the same as in Fig. 2,* i.e., *along the normal to the plane of the caffeine five-membered ring. Arrows in* (b) *indicate the overlapping carbonyl groups.*

The overlap of flat ring systems of barbital and caffeine in the 2:1 complex is restricted to the partial overlap of the five-membered rings of the caffeine molecules with each other and to the overlap of caffeine carbonyl groups with those of barbital molecules (Fig. 3). The caffeine–caffeine interatomic distances such as N(9)···N(9) (3.39 Å) and N(9)···N(7) (3.40 Å) are all longer than the usual van der Waals' distances (Table I), from which it is concluded that these caffeine–caffeine interactions are not significant as a structure-determining influence.

The caffeine carbonyl–barbital carbonyl overlap is of greater interest because the short intermolecular C···O distances (3.02 and 3.04 Å) and the approximately antiparallel C—O stacking resemble the configuration found in the crystal structures of the non-pharmacologically active barbiturates, violuric acid monohydrate (24) and dilituric acid trihydrate (25).

Bolton (26) has pointed out a number of crystal structures, including anhydrous barbituric acid and alloxan, which have intermolecular C═O···C═O distances ranging from as short as 2.77 Å, but with a different geometry such that the C═O bond of one carbonyl group is directed toward the carbon atom of a second. Bolton describes these as dipole–dipole interactions, emphasizing that the carbonyl groups in each case are flanked by electron-withdrawing groups which might be expected to enhance the polar character of the C═O bond.

Prout and Wallwork (27) consider the two different orientations for the carbonyl–carbonyl interactions as examples of a general class of intermolecular dipole–dipole or induced dipole interactions involving polar groups such as C═O and P═O and/or polarizable groups such as aromatic systems and carbon–carbon double bonds. For previously determined xanthine crystal structures, where steric factors permit more extensive molecular overlap, Shefter (12) has summarized results showing a tendency for carbonyl groups to lie over the delocalized π-bonded ring systems of adjacent molecules.

The crystal structure of the 2:1 complex of barbital with caffeine appears to be dominated by barbital–barbital and barbital–caffeine interactions, with NH···O and NH···N hydrogen bonding playing a major role. The C(8)H···O and C═O···C═O interactions may also be important, but the nature of these interactions and the extent to which they impose steric relationships in the environment of the component molecules are not yet well understood. These weaker interactions merit further study because they may enhance drug molecular association in solution and drug binding *in vivo* at sites where polar or polarizable groups are favorably oriented.

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Influences of Heredity and Environment on Alkaloidal Phenotypes in Solanaceae

MARK J. SOLOMON* and FRANK A. CRANE

Abstract □ Modern biometrical analysis of reported data on *Atropa belladonna* L. indicates varying heritabilities at successive stages of plant ontogeny, exhibiting almost complete additivity at the early flowering stage. A discussion of the importance of this observation with accompanying consideration of more general implications is presented.

Keyphrases □ Phenotypes, alkaloidal, in Solanaceae—heredity, environmental influences □ Solanaceae biovariation—selecting for alkaloidal phenotypes, effects of heredity, environment □ Genetic variation, Solanaceae alkaloidal phenotypes—equations, phenotypic scale diagram

Research efforts involving selection for or against alkaloidal phenotypes are relatively rare in the literature. Such studies are lengthy, involved, and relatively wasteful in time and effort in comparison to the information obtained.

Upon reviewing the literature on Solanaceous alkaloidal biovariation, the authors noted that Sievers (1) had grown plants of *Atropa belladonna* L. for economic reasons during World War I and collected data on total alkaloidal content, expressed as milligrams atropine per gram dry weight of powdered leaf. These data lent themselves to heritability analysis (h^2, the potential for selection under artificial or natural conditions), utilizing modern statistical and biometric techniques (2).[1]

The authors' analysis of Sievers' data is summarized in Table I. Since Sievers analyzed parental types and their respective progeny at different stages of ontogeny, the following conclusions may be drawn:

1. Heritability (h^2) is present.
2. This heritability varies with ontogeny, ranging from high values (maximum value of 1) during vegetative and early flowering stages to lower values in early and late fruiting stages.
3. Heritability is maximum at the flowering stage of ontogeny, reaching almost complete additivity.

To the writers' knowledge, this analysis represents the first application of quantitative genetic techniques to tropane alkaloid biovariation, and it is the first example of quantitative estimation of a relationship of plant ontogenesis to a tropane alkaloid.

DISCUSSION

Without the information obtained from Sievers' original data (1) and the authors' later interpretation, four models of genetic control appear possible if workers do not postulate genetic and environmental control of alkaloidal phenotypic variation to occur, as most geneticists would assume. These models are:

1. Alkaloidal phenotypes are not inherited; *i.e.*, they are all environmentally controlled.
2. Alkaloidal phenotypes are inherited in Mendelian fashion; *i.e.*, they are monogenic with no environmental control.
3. Alkaloidal phenotypes are the product of multiple allele segregation (with no environmental control).

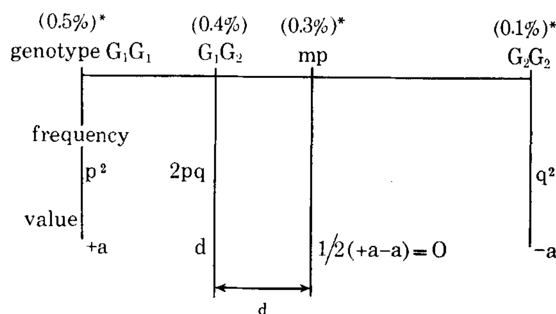

Figure 1—*Alkaloidal phenotypic scale for one gene in the biosynthetic pathway of an alkaloid (26). * A hypothetical case: presence of both dominant alleles would produce a final contribution of 0.5% alkaloidal content. The double recessive G_2G_2 would produce a concentration of 0.1%. If the alleles are additive, the heterozygote A_1A_2 would be 0.3%. If dominance is present, the G_1G_2 genotype would have the value of 0.4%.*

G_1G_1 = *homozygous dominant (maximum contribution to alkaloidal phenotype)*
G_2G_2 = *homozygous recessive (minimum contribution to alkaloidal phenotype)*
G_1G_2 = *heterozygote value (0 point or deviation if dominance is present)*
d = *dominance deviation from midparent*
mp = *midparent value (additive value of G_1G_1 + additive value of G_2G_2 divided by 2)*
α_1 = *average effect when G_1 is substituted for G_2*
α_2 = *average effect when G_2 is substituted for G_1*
α_T = *total average effects on the phenotype*
a = *additive effect due to one allele substitution*
p = *frequency of G_1 allele in population*
q = *frequency of G_2 allele in population*
$p + q$ = *1 since each gene has only two alleles*

[1] The statistical techniques used for this analysis were not known in 1915. It is a tribute to his scientific acumen that Sievers' data could be analyzed over a half-century later to yield these results.