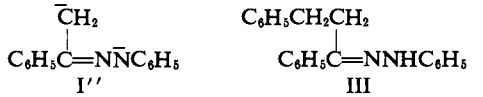
1,4-Dianions of Phenylhydrazones Having an α-Hydrogen[1]

Sir:

Acetophenone phenylhydrazone has recently been converted to the monoanion I′ by 1 equiv of potassium amide in liquid ammonia, as evidenced by N-benzylation with benzyl chloride to form II.[2]

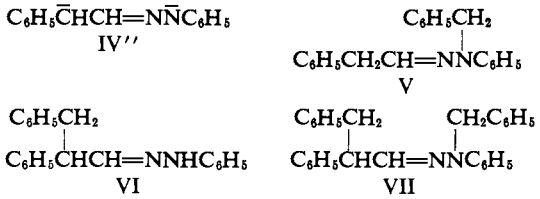
I′ II

This phenylhydrazone has now been converted to the dianion I″ by 2 equiv of the alkali amide in liquid ammonia, as evidenced by preferential C-benzylation with 1 equiv of benzyl chloride to afford III, which was independently synthesized from benzylacetophenone and phenylhydrazine.



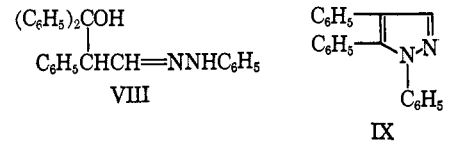
I″ III

Similarly, phenylacetaldehyde phenylhydrazone was N-benzylated through its monoanion and C-benzylated through its dianion IV″ to form V and VI, respectively. Also, dianion IV″ underwent dibenzylation with 2 equiv of benzyl chloride to give VII.



IV″ V VI VII

Likewise, dianion IV″ underwent an addition reaction with benzophenone and benzoylation, accompanied by cyclization with methyl benzoate, to afford VIII and IX, respectively.



VIII IX

The yields of all of these products were good (50–76%). The structures of the new compounds III, V, VI, VII, and VIII were supported by analyses and infrared and nmr spectra. Cyclic structure IX was supported by infrared and by essential agreement of melting point with the values reported previously.[3] The present method not only affords a better yield but also appears more convenient.

These results represent a significant advance in our studies of dianions, the two anionic portions of which are in resonance.[4] Dianions I″ and IV″ are to be distinguished from ordinary 1,4-dianions in which the two anionic portions are not in resonance. Because the carbanion portion of I″ or IV″ is much more nucleophilic, C-condensations can be effected to the apparent exclusion of N-condensations. However, C- and N-condensations might be realized to form cyclic products with appropriate electrophilic compounds.

Work is in progress on condensations of dianions I″ and IV″ with other electrophilic compounds and on similar studies with various other phenylhydrazones including osazones and related compounds.

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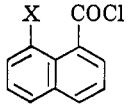
(1) Supported by the Petroleum Research Fund, administered by the American Chemical Society, and the National Science Foundation.
(2) W. G. Kenyon and C. R. Hauser, *J. Org. Chem.*, **30**, 292 (1965).
(3) W. Wislicenus and A. Ruthing, *Ann.*, **379**, 229 (1911); J. Matti and M. Perrier, *Bull. Soc. Chim. France*, **22**, 525 (1955); H. O. House and D. Ryerson, *J. Am. Chem. Soc.*, **83**, 979 (1961).
(4) Several 1,3-dianions, such as that of acetylacetone, have previously been prepared; see C. R. Hauser and T. M. Harris, *J. Am. Chem. Soc.*, **80**, 6360 (1958).

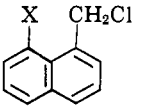
Relative Contributions of Hyperconjugation and Nonbonded Interactions to Secondary Isotope Effects

Sir:

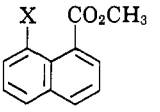
Solvolytic rate constants and isotope effects for compounds I–III[1] are summarized in Table I. The



Ia, X = CH_3
b, X = CD_3
c, X = H
d, X = D



IIa, X = CH_3
b, X = CD_3
b′, (−CD_2Cl, X = CH_3)
c, X = H
d, X = D
d′, (−CD_2Cl), X = H

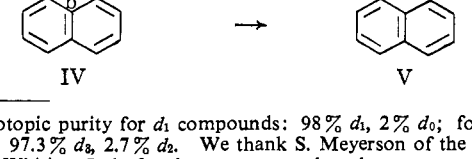


IIIa, X = CH_3
c, X = H
d, X = D

absence of formal hyperconjugative structures involving the isotopically labeled group X dictated the study of these compounds as a probe into the still debated question of the relative contributions of hyperconjugation and nonbonded interactions to secondary isotope effects.

Since k_{Ia}/k_{Ic} is about 10, whereas the nucleophilic k_{IIIa}/k_{IIIc} is less than 10^{-3}, Ia must solvolyze by a limiting mechanism. As judged from k_{IIa}/k_{IIc} of about 30 and an α-isotope effect $k_{IIa}/k_{IIb'}$ of 1.32, IIa also solvolyzes by a limiting mechanism. That in formic acid IIc solvolyzes by a limiting mechanism has been established[2] and supported here by the α-isotope effect $k_{IIc}/k_{IId'}$ of 1.35.

From currently available potential functions,[3,4] $V(r)$, and using Bartell's procedure,[3] we have calculated and summarized in Table II the nonbonded isotope effect (k_{Ia}/k_{Ib}) for IV → V as a function of the dihedral



IV V

(1) Isotopic purity for d_1 compounds: 98% d_1, 2% d_0; for d_3 compounds: 97.3% d_3, 2.7% d_2. We thank S. Meyerson of the American Oil Co., Whiting, Ind., for the mass spectral analyses.
(2) M. J. S. Dewar and R. J. Sampson, *J. Chem. Soc.*, 2789 (1956); 2946 (1957).
(3) L. S. Bartell, *J. Am. Chem. Soc.*, **83**, 3567 (1961).
(4) R. A. Scott and H. A. Scheraga, *J. Chem. Phys.*, **42**, 2209 (1965). For the H ↔ C interaction the function given by J. B. Hendrickson, *J. Am. Chem. Soc.*, **83**, 4537 (1961), was used.

Table I. Rate Constants and Isotope Effects for I–III at 25.00°

Compd	Solvent[a]	Mechanism	$k \times 10^5$, sec^{-1}	k_H/k_D, 25°	$\Delta\Delta G^{\ddagger}$,[b] cal/mole
Ia	A	Lim	46.75 ± 0.59		
Ib	A	Lim	45.44 ± 0.34	1.029 ± 0.015	−16.6 ± 8.3
Ic	A	Unknown	5.44 ± 0.12		
Ic	B	Unknown	17.99 ± 0.18		
Id	B	Unknown	18.09 ± 0.24	0.99 ± 0.014	+5.9 ± 8.2
IIa	C	Lim	0.3126 ± 0.0045		
IIb	C	Lim	0.3086 ± 0.0028	1.013 ± 0.024	−7.7 ± 14
IIb′	C	Lim	0.2366 ± 0.0004	1.321[c] ± 0.021	−165 ± 9
IIc	C	Unknown	0.0099 ± 0.0005		
IIc	D	Lim	4.49 ± 0.11		
IId	D	Lim	4.51 ± 0.04	1.00 ± 0.05	0 ± 29
IId′	D	Lim	3.32 ± 0.02	1.35[c] ± 0.05	−178 ± 23
IIIa	E	Nucl	<0.1[d]		
IIIc	E	Nucl	311 ± 2.0[d]		
IIId	E	Nucl	311 ± 0.9[d]	1.00 ± 0.01	0 ± 6

[a] A, 95% (w/w) acetone–water. B, 90% (w/w) acetone–water. C, 61.4% (w/w) acetone–water. D, 0.32 M in water in formic acid. E, 56% (w/w) acetone water. The base and ester concentrations were 5×10^{-2} M. [b] $\Delta\Delta G^{\ddagger} = \Delta G_H^{\ddagger} - \Delta G_D^{\ddagger}$. [c] α-isotope effect. [d] Second-order rate constants in liters per mole per second.

Table II. Calculated k_{Ia}/k_{Ib} from Nonbonded Potential Functions, $V(r)$

		$V(r)$[b]		$V(r)$[c]	
ϕ, deg	ϕ', deg	$\Delta\Delta E$,[a] cal/mole	k_{Ia}/k_{Ib}, 25°	$\Delta\Delta E$,[a] cal/mole	k_{Ia}/k_{Ib}, 25°
0	0	−870	4.31	−530	2.43
15	7.5	−710	3.32	−420	2.03
30	14	−460	2.17	−230	1.48
45	18	−250	1.52	−100	1.18
60	20	−116	1.21	−40	1.07
75	20	−90	1.16	−20	1.03
90	0	−81	1.15	−38	1.03

[a] $\Delta\Delta E = \Delta E_H - \Delta E_D$. [b] From ref 3. $\Delta\Delta E$ calculated as outlined in ref 3. [c] From ref 4. $\Delta\Delta E = 0.135 l_m^2[V'' + {}^1/_2 l_t^2 V^{IV}]$. In both *b* and *c* the values used (angstroms) were: $l_m = 0.09$, $l_t = 0.16$ for H ↔ Cl and H ↔ O interactions; $l_m = 0.10$, $l_t = 0.14$ for H ↔ C interactions.

ring and the nitro groups of 1,8-dinitronaphthalene[6] and 1,5-dinitonaphthalene[7] are 43 and 49°, it is reasonable to assume that ϕ is between 45 and 60°. The isotope effects, therefore, especially those calculated using Bartell's functions, are higher than the experimental.

In Table III we have summarized a few calculated and experimental isotope effects that cogently illustrate the points that we want to emphasize with respect to the relative contributions of hyperconjugation and nonbonded interactions. Use of the Scott and Scheraga potential functions[4] leads to reasonable estimates of the α-isotope effect, which is overestimated from Bartell's potential functions, and to the best values for k_{Ia}/k_{Ib}. In cases, however, where

Table III. Comparison of Calculated and Experimental Secondary Isotope Effects for Some Limiting Mechanism Solvolyses

Compd	$\Delta\Delta E$[a] calcd, cal/mole	k_H/k_D calcd, 25°	$V(r)$ from ref	$\Delta\Delta G^{\ddagger}$[b] exptl, cal/mole	k_H/k_D exptl, 25°	Ref
RCD_2Cl[c]	−154	1.30	4		1.30	*d*
	−592	2.73	3			
CD_3COCl[e,i]	+8.1	0.98 (−22°)	4	−240 ± 25	1.62 ± 0.08	*f*
	−8.6[h]	1.02 (−22°)	4			
	+29	0.94 (−22°)	3			
	−38[h]	1.08 (−22°)	3			
$(CD_3)_3CCl$	−59	1.10	4	−516	2.39	*g*
	−179	1.35	3			
	(−198)[i]	(1.39)	3			

[a] $\Delta\Delta E = \Delta E_H - \Delta E_D$. [b] $\Delta\Delta G^{\ddagger} = \Delta G_H^{\ddagger} - \Delta G_D^{\ddagger}$. [c] Calculated fort he CD_2Cl fragment only. Values (angstroms) used: $l_m = l_t = 0.135$ for H ↔ H interactions, from Y. Morino, K. Kuchitsu, A. Takahashi, and K. Maeda, *J. Chem. Phys.*, **21**, 1927 (1953); $l_m = l_t = 0.104$ for H ↔ Cl interactions, from J. M. Hastings and S. H. Bauer, *ibid.*, **18**, 13 (1950). [d] Several investigations including this work. [e] Structures for acetyl chloride and cation from K. M. Sinnott, *J. Chem. Phys.*, **34**, 851 (1961), and F. P. Boer, *J. Am. Chem. Soc.*, **88**, 1572 (1966), respectively. [f] M. L. Bender and M. S. Feng, *ibid.*, **82**, 6318 (1960). [g] L. Hakka, A. Queen, and R. E. Robertson, *ibid.*, **87**, 161 (1965). [h] Not taking into account H ↔ C interactions. [i] Value reported in ref 3. [j] Values (angstroms) used: $l_m = 0.10$, $l_t = 0.18$ for H ↔ Cl interactions; and $l_m = l_t = 0.10$ for H ↔ C interactions.

angle ϕ between the ring and COCl planes. The angles *a*, *b* and *c* were taken as 123.5, 126.8, and 123.5° to conform with similar systems.[5] ϕ' is the angle between the H_1CC and ring planes in IV; it was calculated as a function of ϕ by maximizing the nonbonded distances in Ia.

To assess the reliability of the calculated isotope effect, a reasonable estimate of ϕ is required. From the fact that the dihedral angles between the planes of the hyperconjugation is possible, *e.g.*, acetyl chloride and *t*-butyl choride, these functions[4] account for less than 10% of the experimental isotope effect.[8]

(5) M. B. Janeson and B. R. Penfold, *J. Chem. Soc.*, 528 (1965).

(6) Z. A. Akopyan, A. I. Kitaigorodskii, and Yu. T. Struchov, *J. Struct. Chem.* (USSR), **6**, 690 (1965).

(7) J. Trotter, *Acta Cryst.*, **13**, 95 (1960).

(8) Even invoking nonbonded relief through charge delocalization,[8] which is "tantamount to invoking hyperconjugation,"[8] does not remedy the situation. This effect, which we shall call a "second-order" nonbonded isotope effect, plus the "first-order" effect account for about half of the experimental isotope effect if the Scott and Scheraga potential functions are used.

It should be emphasized that "hardening" the potential functions to account for the observed isotope effect in acetyl chloride and *t*-butyl chloride inevitably leads to grossly overestimated effects in systems where hyperconjugation is impossible.

In summary, we conclude: (1) in ordinary systems with hyperconjugation possible, less than 10%—probably 2–5%—of the observed isotope effect is due to nonbonded interactions; reasonable estimates of the nonbonded isotope effect might be obtained by using Bartell's procedure with the Scott and Scheraga functions.

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(9) National Institutes of Health Predoctoral Fellow, March 15–Sept 30, 1966.

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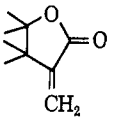
The Isolation and Structural Elucidation of Euparotin Acetate, a Novel Guaianolide Tumor Inhibitor from *Eupatorium rotundifolium*[1,2]

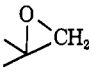
Sir:

In the course of a continuing search for tumor inhibitors of plant origin, alcoholic extracts of *Eupatorium rotundifolium* L. (Compositae)[3] showed significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB).[4] We report herein the isolation and structural elucidation of euparotin acetate, a novel tumor-inhibitory sesquiterpene of the guaianolide type from *E. rotundifolium*.

Fractionation of the ethanol extract, guided by assay against KB, revealed that the active principles were concentrated, successively, in the chloroform layer of a chloroform–water partition and in the aqueous methanol layer of a 10% aqueous methanol–petroleum ether partition. Further fractionation involving silicic acid chromatography yielded euparotin acetate (III),[5] $C_{22}H_{26}O_8$: mol wt (mass spectroscopy),[6] 418; mp 156–157° (vac); $[\alpha]^{30}D$ −191° (*c* 0.54, EtOH); λ_{max}^{EtOH} end absorption at 210 mμ (ε 18,400); λ_{max}^{KBr} 2.91, 5.67, 5.74, 5.85, 6.03, 6.04, 6.06, 7.49, 7.95, 8.90, 9.75, 10.20 μ; and nmr signals (in $CDCl_3$) at τ 3.62 and 4.33 (2 H, doublets, J = 3.5 cps, I), 3.93 (1 H, q, J = 7 cps, vinyl H), 4.28 (3 H, multiplets, vinyl H and 2 >CH–O), 5.18 (1 H, d, J = 8 cps, >CH–O), 5.78 (1 H, m), 7.12 (1 H, br s, OH), 7.30 (2 H, s, II), 7.97 (3 H, s, –O–$COCH_3$), 8.02 and 8.18 (9 H, multiplets, vinyl methyls).



I



II

Further chromatography yielded euparotin (IV), $C_{20}H_{24}O_7$: mol wt (mass spectroscopy),[6] 376; mp 199–200° (vac); $[\alpha]^{32}D$ −124° (*c* 1.25, EtOH); λ_{max}^{EtOH} end absorption at 213 mμ (ε 17,800); λ_{max}^{KBr} 2.90, 5.68, 5.86, 6.05, 6.08, 6.17, 7.98, 8.73, 9.88 μ; and nmr signals (in $CDCl_3$) at τ 3.61 and 4.36 (2 H, doublets, J = 3.5 cps, I), 3.96 (1 H, q, J = 7 cps, vinyl H), 4.26 (1 H, m, vinyl H), 5.17 (1 H, d, J = 8 cps, >CH–O), 5.17 and 5.87 (2 H, multiplets), 7.28 (2 H, s, II), 8.04 and 8.20 (9 H, multiplets, vinyl methyls). Acetylation of IV gave III.

Acylation of euparotin with bromoacetic anhydride gave the bromoacetate V. Euparotin bromoacetate crystallized from benzene–petroleum ether as a benzene solvate, $C_{22}H_{25}BrO_8 \cdot 0.5C_6H_6$, mp 156–157°, $[\alpha]^{32}D$ −142° (*c* 0.38, EtOH), the crystals of which belong to the monoclinic system, space group C2, with four units of $C_{22}H_{25}BrO_8 \cdot 0.5C_6H_6$ in a cell of dimensions a = 34.85, b = 7.04, c = 10.90 A, β = 106° 35′; 1947 independent $|F_0|$ values were derived from the three-dimensional X-ray intensity data which were recorded on equiinclination Weissenberg photographs and visually estimated.

The initial position of the bromine atom was obtained from the three-dimensional Patterson synthesis. The carbon and oxygen atoms were located in three-dimensional electron-density distributions for which the Fourier coefficients were weighted according to the method proposed by Sim.[7] The atomic coordinates were subsequently refined by the least-squares method and the present value of R is 13.1%. The bromine atom was assigned anisotropic temperature factors, but the carbon and oxygen atoms were assigned only isotropic values.

The results of the X-ray analysis establish that the bromoacetate has structure V and it follows, therefore, that euparotin has structure IV, and euparotin acetate, structure III. The absolute configuration was deduced by Bijvoet's anomalous dispersion method.[8]

Euparotin appears to be the most highly oxygenated guaianolide reported to date (*i.e.*, six oxygen functions in the basic C_{15} nucleus) and the first recognized to contain a spiroepoxide.[9] It is noteworthy that euparotin

(1) Tumor Inhibitors. XX. Part XIX: S. M. Kupchan, R. J. Hemingway, and J. C. Hemingway, *J. Pharm. Sci.*, in press.

(2) Supported by grants from the National Cancer Institute (CA-04500), the American Cancer Society (T-275), the National Science Foundation (GB-2878), and a contract with the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health (PH 43-64-551).

(3) Leaves, stems, flowers, and roots were gathered in Florida, Sept 1960. The authors acknowledge with thanks receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md., in accordance with the program developed with the U. S. Department of Agriculture by the Cancer Chemotherapy National Service Center.

(4) Cytotoxicity and *in vivo* inhibitory activity were assayed under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, by the procedures described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

(5) Euparotin and euparotin acetate showed significant cytotoxicity (ED_{50}) against KB (human carcinoma of the nasopharynx) cell culture at 0.21 μg/ml. Euparotin acetate showed significant inhibitory activity against Walker carcinosarcoma 256 in rats at 75 mg/kg.[4]

(6) The authors thank Professor A. L. Burlingame and Dr. H. K. Schnoes, University of California, Berkeley, for the mass spectral data.

(7) G. A. Sim, *Acta Cryst.*, **12**, 813 (1959); **13**, 511 (1960); "Computing Methods and the Phase Problem in X-ray Crystal Analysis," R. Pepinsky, J. M. Robertson, and J. C. Speakman, Ed., Pergamon Press, Oxford, 1961, p 227.

(8) J. M. Bijvoet, A. F. Peerdeman, and A. J. van Bommel, *Nature*, **169**, 271 (1951).

(9) For a comprehensive review, see F. Sorm and L. Dolejš, "Guaianolides and Germacranolides", Editions Scientifiques Hermann, Paris, 1966.