icantly increased abnormalities was 300 μg or more. The last dose of LSD-25 taken by six of the eight users before sampling was betweeen 1 to 30 days; two of the subjects exhibiting abnormalities took theirs 3 and 6 months earlier, respectively. One of the LSD-25 subjects exhibiting abnormalities had had only four doses of approximately 300 μg each.

Table 1. Number and type of chromosomal breaks in controls and LSD-25 users. Data are given as total number of breaks in 200 cells classified.

Case No.	Breaks (No.)		Exchange figures	Chromosomes	
	Chromatid	Isochromatid		Dicentric	Rings
		Controls			
12-F	9	3			1
14-M	13	1			
4-M	14	3			
16-M	16	3			
11-F	17	3			
3-M	18	3			
8-M	20	1		3	
6-F	29	4			
13-M	38	12			
		LSD-25			
1-M	15	5		2	
7-M	21	2	1		
17-M	35	7			
10-M	39	5*		1	
20-M	36	10	2	1	
15-M	42	8			
5-M	58	5			
9-M	61	9*	1	2	

* Ph_1-like chromosomes.

Table 2. Distribution of abnormal cells and chromosomal breaks in controls and LSD-25 users as related to number of doses and dosage. A total of 200 cells were classified for each subject. $P < .05$ for abnormal cells and breaks.

Case No.	Doses (No.)	Estimated dose (μg)		Last dose (days)	Abnormal cells (%)	Breaks (%)
		Mean	Peak			
			LSD-25			
1-M	10	200	800	14	11.0	12.0
7-M	100	200	500	14	12.0	12.5
17-M	4	300	400	30	21.0	21.0
10-M	50	300	1500	180	21.0	23.0
20-M	50	500	1500	3	24.5	26.0
15-M	110	350	600	90	25.0	25.0
5-M	25	400	500	21	30.0	31.5
9-M	200	350	2800	1	34.0	38.0
			Controls			
12-F					6.5	7.0
14-M					7.0	7.0
4-M					8.0	8.5
16-M					9.5	9.5
11-F					9.5	10.0
3-M					10.0	10.5
8-M					11.5	13.5
6-F					14.0	16.5*
13-M					24.0	25.0†

* Recent diagnostic x-ray. † Past x-ray therapy.

Figure 1 illustrates some of the abnormalities seen among the LSD-25 users. Of special interest were the quadriradial formations (three cases); these occur rarely in untreated, normal cultures (*4*) but can be induced in human leukocytes by the addition of mitomycin (*5*). Quadriradials and increased chromosomal breaks also are characteristic of the cytogenetic picture of Fanconi's anemia and Bloom's syndrome (*6*), both caused by recessive autosomal genes and accompanied by an increased incidence of neoplasia (*7*). They are also seen in tumor cells or cells transformed by the oncogenic virus SV40 (*8*). The genetic consequences of the quadriradial figures have been generally discussed (*4, 9*).

Also of much interest is the Ph_1-like chromosome observed in LSD-25 users 9 and 10. The Ph_1 chromosome, a deleted G-group autosome, is characteristic of chronic myelogenous leukemia (*10*) and is thought to be absent from lymphoid cells but present in neutrophil and erythrocyte precursors and possibly megakaryocytes of the bone marrow (*11*). All cells showing the Ph_1-like chromosome were karyotyped; in every instance, only three normal G-group chromosomes plus the Ph_1-like chromosome were found.

It is still too early to assess the significance of these findings. The chromosomal abormalities in human lymphocytes, for the moment, appear to be induced by as little as four doses of LSD-25 exceeding an estimated 200 μg. It remains to be determined also whether the chromosomal abnormalities result from permanent damage to the stem cells, or from damage in the G_1 period to long-lived lymphocytes, the damage not being recognized as chromosomal abnormalities until mitosis.

SAMUEL IRWIN

Department of Psychopharmacology, Oregon Regional Primate Research Center, and *Department of Psychiatry, University of Oregon Medical School, Portland*

JOSE EGOZCUE

Department of Genetics, Oregon Regional Primate Research Center, Beaverton

References and Notes

1. M. M. Cohen, M. J. Marinello, N. Back, *Science* **155**, 1417 (1967).
2. J. Egozcue and M. Vilarasau de Egozcue, *Stain Technol.* **41**, 173 (1966).
3. K. E. Buckton, P. A. Jacobs, W. M. Court-Brown, R. Doll, *Lancet* **1962-II**, 676 (1962); J. Visfeldt, *Acta Radiol.* **2**, 95 (1964); M. M. Nofal and W. H. Beierwaltes, *J. Nucl. Med.* **5**, 849 (1964); W. D. Macdiarmid, *Quart. J. Med.* **34**, 133 (1965); A. Dekaban, *J. Nucl. Med.* **6**, 740 (1965).
4. J. German, *Science* **144**, 298 (1964).
5. M. M. Cohen and M. W. Shaw, *J. Cell Biol.* **23**, 386 (1964).
6. J. German, R. Archibald, D. Bloom, *Science* **148**, 506 (1965); G. E. Bloom, S. Warner, P. S. Gerald, L. K. Diamond, *New Engl. J. Med.* **274**, 8 (1966).
7. S. Garriga and W. H. Crosby, *Blood* **14**, 1008 (1959); G. E. Bloom, S. Warner, P. S. Gerald, L. K. Diamond, *New Engl. J. Med.* **274**, 8 (1966); A. Sawitsky, D. Bloom, J. L. German, *Ann. Internal Med.* **65**, 486 (1966).
8. T. C. Hsu and G. K. Manna, *Amer. Natural.* **93**, 207 (1959); M. Fraccaro, A. Mannini, L. Tiepolo, C. Zara, *Mutation Res.* **2**, 559 (1965); S. R. Wolman, K. Hirschhorn, G. J. Todaro, *Cytogenetics* **3**, 45 (1964).
9. M. W. Shaw and M. M. Cohen, *Genetics* **51**, 181 (1965).
10. P. C. Nowell and D. A. Hungerford, *J. Nat. Cancer Inst.* **28**, 85 (1960).
11. J. M. Trujillo and S. Ohno, *Congr. Int. Soc. Hematol. 9th Mexico* (Universidad Nacional Autonoma de Mexico, Mexico City, 1962), vol. 1, p. 305; I. M. Tough, P. A. Jacobs, W. M. Court-Brown, A. G. Baikie, E. R. D. Williamson, *Lancet* **1963-I**, 844 (1963); J. Whang, E. Frei, III, J. H. Tjio, P. P. Carbone, G. Brecher, *Blood* **22**, 664 (1963).
12. Publication No. 244 of the Oregon Regional Primate Research Center. Supported in part by NIH grant F00163 and in part by NIMH grant MH-12214. We thank F. Hagemenas for technical assistance.

10 May 1967

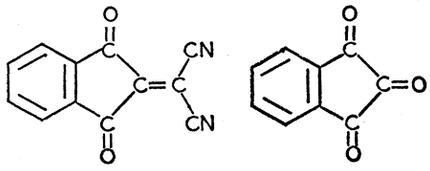
2-Dicyanomethylene-1,3-Indanedione: A New Electron Acceptor

Abstract. *The electron affinity of 2-dicyanomethylene-1,3-indanedione (I) (1.33 ev), as computed from charge-transfer spectral analysis and from polarographic measurements of half-wave reduction potential, shows that the incorporation of the 2-dicyanomethylene moiety in 1,2,3-indanetrione (II) enhances its π-acid character. The electron spin resonance spectrum of the anion radical of I, generated electrolytically in solution, shows hyperfine structure that is due to two nitrogen atoms and four equivalent hydrogen atoms.*

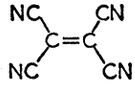
The acceptor strength of a π-electron system is based on the electron-withdrawing power, number, and location of the substituents as well as on the extent of conjugation. The π-acid strength and electron affinity of an acceptor change in a predictable manner when one or more of the above factors are systematically varied (*1*). The suitable combination of two acceptor moieties within one molecular framework could possibly lead to an acceptor which might possess a greater electron affinity than molecules containing only one of the parent moieties (*2*).

We studied the electron affinity and π-acid property of a new electron ac-

ceptor, 2-dicyanomethylene-1,3-indanedione (I), which incorporates the structural features of the two acceptors, 1,2,3-indanetrione (II) (*3*) and tetracyanoethylene (III) (*4*).



I II



III

Compound I was obtained as yellow crystals from 1,2,3-indanetrione (II) and malononitrile (m.p. 280° to 285°C). It showed absorption maxima at 261 mμ in CH_3CN [molar extinction (ε) being 27,600], at 271 mμ (ε being 28,700), and at 350 mμ (ε being 6600). In KBr there was an absorption maximum at 4.50 μ, an indication of the presence of a conjugated nitrile structure, and at 5.95 μ, an indication of a conjugated ketone structure (*5*).

In dichloromethane solution, compound I generates charge-transfer complexes readily with aromatic hydrocarbons. The charge-transfer bands appear in the visible region between 450 and 750 mμ (Table 1). To estimate the electron affinity of compound I, we used the relation (*6*)

$$E_\pi = I_p - E_A + C \qquad (1)$$

where E_π is the charge-transfer transition energy, I_p is the ionization potential of the donor, and C is a constant-energy term which represents the effects of solvation, polarization, and nonbonding interactions. For the complexes of two acceptors with the same series of donors, C is generally assumed constant as long as the same type of bonding (for example, π-π) is involved. Under these circumstances

$$E_\pi + E_A = E'_\pi + E'_A \qquad (2)$$

where E'_π is the charge-transfer transition energy of a standard acceptor taken here as chloranil (E'_A, 1.37 ev) (*1*, *7*). The electron affinity was computed from the E_π values for compound I and E'_π value for chloranil (Table 1). The average of E_A is 1.33 ev.

The reversible polarographic reduction potential ($E_{\frac{1}{2}}$) of an acceptor has been related to the energy of the lowest unfilled orbital of the acceptor, and hence to its electron affinity (*8*). When expressed relative to the potential of the saturated calomel electrode, the pertinent relation is

$$-E_{\frac{1}{2}}^{red} = E_A - \Delta F_{solv} - 5.07 \qquad (3)$$

where $-\Delta F_{solv}$ is the solvation energy of the anion and is assigned an average value of −3.66 ev for a number of acceptors having analogous structures. The polarographic reduction of compound I in acetonitrile ($10^{-3}M$) containing $0.1M$ tetra-*n*-propyl ammonium perchlorate (*9*) showed a single wave with $E_{\frac{1}{2}} = +0.02$ volt. Analysis of the polarogram with the Nernst equation indicated that this half-wave potential corresponds to a reversible reduction involving one electron. The computed electron affinity, $E_A = 1.38$ ev, agrees well with the value obtained from the charge-transfer spectra. Under similar conditions, 1,2,3-indanetrione (II) gave $E_{\frac{1}{2}} = 0.47$ volt and $E_A = 0.97$ ev (*10*). This value is also in agreement with the $E_A = 1.07$ ev obtained from charge-transfer spectral measurements (*3*).

Table 1. Charge-transfer spectra (E_π) and electron affinity (E_A) of compound I. Absorption maximum, λ_{max}.

Hydrocarbon	Compound I		Chloranil*	
	λ_{max} (mμ)	E_π (ev)	E_A (ev)	E'_π (ev)
Hexamethylbenzene	510	2.43	2.39	1.33
Naphthalene	475	2.61	2.49	1.25
Anthracene	647	2.03	1.93	1.27
Phenanthrene	462	2.69	2.63	1.31
1,2-Benzanthracene	600	2.07	2.11	1.41
Pyrene	615	2.11	2.00	1.36
3,4-Benzpyrene	700	1.80	1.75	1.32
Perylene	755	1.64	1.63	1.36
Coronene	600	2.07	2.12	1.42

* See reference *12*.

Although I have been unable to isolate solid anion radical complexes of compound I, these species have been generated in solution. For this purpose, the compound was allowed to react with sodium in degassed dimethoxyethane. The solution shows strong absorption at 400, 625, and 940 mμ. The electron spin resonance spectrum (*11*) of this complex consists of only five lines with a line separation of 0.87 gauss. Relative intensities in the ratio 1 : 2 : 3 : 2 : 1 showed clearly that the nitrogen atoms are equivalent with respect to splitting. By contrast, the electrolysis of compound I in degassed mixture of acetonitrile and dimethoxyethane containing $0.1M$ tetra-*n*-propylammonium perchlorate (*11*) shows a well-separated 25-line splitting pattern arising from two nitrogen atoms (a_N, 0.85 gauss) and four equivalent protons (a_H, 0.25 gauss) (Fig. 1).

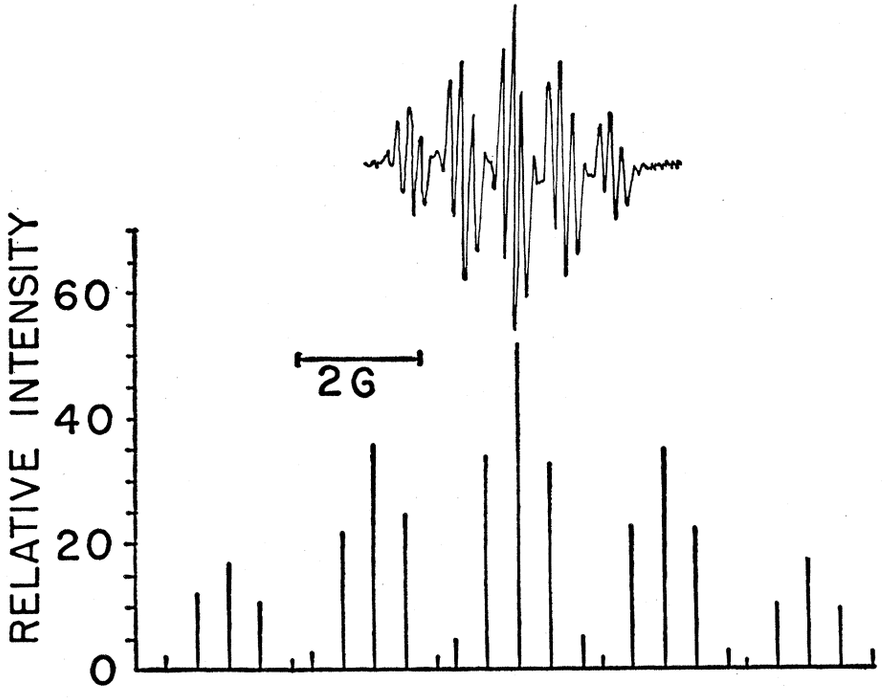


Fig. 1. Electron spin resonance spectrum of electrolytically generated anion radical of I ($10^{-4}M$) in 80 percent of CH_3CN and 20 percent of dimethoxyethane, with $0.1M$ tetra-*n*-propylammonium perchlorate.

The acceptor strength of the present compound is intermediate between that of the related but weaker π-acid, 1,2,3-indanetrione (II) and that of tetracyanoethylene (III) ($E_A = 1.70$ ev).

SUPRABHAT CHATTERJEE
Air Force Cambridge Research Laboratories, L. G. Hanscom Field, Bedford, Massachusetts 01730

References and Notes

1. G. Briegleb, *Angew. Chem. Int. Ed. Engl.* **3**, 617 (1964); *Electron-Donator-Acceptor-Komplexe* (Springer-Verlag, Berlin, 1961).
2. P. R. Hammond, *Science* **142**, 502 (1963).
3. A. R. Lepley and J. P. Thelman, *Tetrahedron* **22**, 101 (1966).
4. R. E. Merrifield and W. D. Philips, *J. Amer. Chem. Soc.* **80**, 2778 (1958).
5. See *Abstracts*, 153rd Meeting, Amer. Chem. Soc., Miami Beach, Florida, 1967, Sect. O, 15.
6. H. McConnell, J. S. Ham, J. R. Platt, *J. Chem. Phys.* **21**, 66 (1953).
7. M. Bately and L. E. Lyons, *Nature* **196**, 573 (1962).
8. A. Maccoll, *ibid.* **163**, 178 (1949); R. M. Hedges and F. A. Matsen, *J. Chem. Phys.* **28**, 950 (1958); M. E. Peover, *Trans. Faraday Soc.* **58**, 1656 (1962).
9. Solvent was dried before use. The polarographic measurements were made with a Sargent polarograph Model 21, a Sargent IR Compensator Model A, and a dropping-mercury electrode.
10. In Eq. 3, $E_{\frac{1}{2}}$ is taken as positive.
11. Measurements of ESR were taken with a standard Varian V-4500 spectrometer (with a modulation of 100 kc/sec) and a magnet (9 cm). Electrolysis was conducted between platinum and silver-silverperchlorate reference electrodes at 1.0 to 1.12 volts.
12. R. Beukers and A. Szent-Györgyi, *Rec. Trav. Chim.* **81**, 541 (1962).
13. I thank Dr. K. Bower (M.I.T.) for providing the electrolytic cell, and Professor K. Weiss (Northeastern University) for suggestions.

24 May 1967

Lungfish Neoceratodus forsteri: Activities of Ornithine-Urea Cycle and Enzymes

Abstract. *The level of activity of the ornithine-urea cycle is low in the liver of the permanently aquatic Australian lungfish. The rate of incorporation of ^{14}C-bicarbonate into urea by liver slices was only 100th of that previously observed in the estivating African lungfish* Protopterus dolloi. *The activities of enzymes of the ornithine-urea cycle were similarly reduced. The low activity of this cycle in* Neoceratodus *is consistent with its exclusively aquatic nature.*

Members of the two surviving families of lungfishes, Lepidosirenidae and Ceratodontidae, differ from one another in ecological behavior. The African *Protopterus* and the South American *Lepidosiren*, belonging to the former family, must breathe air at rather frequent intervals and they estivate in burrows out of water during dry seasons. The sole surviving member of the latter family, the Australian *Neoceratodus forsteri*, appears to use its lung only as an accessory respiratory organ during periods of high activity, and it cannot survive deprivation of water by burrowing and breathing air as can the African and South American dipnoans (*1*). Ability of the liver to synthesize urea by way of the ornithine-urea cycle was recently demonstrated in the African lungfish *Protopterus* (*2, 3, 4*). This cycle has survival value in *Protopterus* during its periods of estivation, when ammonia cannot be excreted and is incorporated into urea. We tried to determine whether *Neoceratodus*, which never leaves the water and therefore has no need to detoxify ammonia, possesses a functional ornithine-urea cycle.

Two adult *Neoceratodus* were obtained from Australia (*5*). The fish, weighing 2.8 and 5.4 kg and in good condition, were immediately placed in separate fiberglass tanks containing aged tap water at 20° to 30°C. During the next 2 days samples of the water were analyzed for ammonia (*6*) and urea (*7*). The rates of excretion of ammonia and urea in fish *X* were 98 and 5.5 μmole kg^{-1} hour^{-1}, respectively. In fish *Y* the rate of excretion of ammonia was 156 μmole kg^{-1} hour^{-1}; no urea was detectable in the bath water. *Neoceratodus* appears to be much more "ammoniotelic" than *P. ethiopicus*, which eliminates up to 50 percent of its nitrogenous end products as urea when in water (*8*).

Within 2 days of arrival the animals were pithed and the livers were removed immediately and assayed for (i) the presence of a functional ornithine-urea cycle in liver slices, and (ii) the individual enzymes of the cycle. Urea-cycle activity of liver slices was assayed by following the incorporation of ^{14}C-bicarbonate into urea (Table 1; *4*). The activities of carbamoyl phosphate synthetase, ornithine carbamoyltransferase (*Enzyme Nomenclature*, 2. 1.3.3), argininosuccinate synthetase (6. 3.4.5), argininosuccinate lyase (4.3.2.1), and arginase (3.5.3.1) were assayed in liver homogenates according to Janssens and Cohen (Table 1; *3*). Carbamoyl phosphate synthetase, being barely detectable by the colorimetric assay, was assayed by another method (*9*). Enzyme activities were calculated from the ^{14}C incorporated into citrulline, relative to the specific activity (counts per minute per micromole) of the ^{14}C-bicarbonate substrate.

The percentage incorporation of ^{14}C-bicarbonate into urea was very low (Table 1), being 0.01 to 0.02 percent of the radioactivity present in the medium —about 100 count/min (above background) as ^{14}C-urea. The average production of ^{14}C-urea by liver slices from both fish was 480 count/min g^{-1} hour^{-1}. The boiling of liver slices for 5 minutes reduced the incorporation of ^{14}C-bicarbonate into urea to one-tenth of the value observed in untreated slices (fish *X*, Table 1). Addition of unlabeled citrulline to the incubation medium, which was expected to reduce the ^{14}C incorporated into urea by competing with ^{14}C-citrulline formed from ^{14}C-bicarbonate, had little effect on the rate of synthesis of ^{14}C-urea. On the other hand, unlabeled arginine reduced the rate of synthesis of ^{14}C-urea to about one-third of that for untreated slices.

The difference in effectiveness of unlabeled citrulline and arginine as inhibitors of synthesis of ^{14}C-urea may be related to relative rates of uptake or to differences in pool size of the two

Table 1. Incorporation of ^{14}C-labeled bicarbonate into urea by liver slices of *Neoceratodus*. Approximately 200-mg slices were incubated in 3.0 ml of Krebs-Ringer solution (diluted 2 parts to 3 with water) containing $10^{-2}M$ ammonium chloride, $10^{-2}M$ sodium lactate, $10^{-3}M$ L-ornithine, and ^{14}C-labeled bicarbonate (6.8×10^5 count/min). The mixtures were shaken for 1 hour in 100-percent oxygen at 30°C.

Fish	Modification of assay	Incorporation (%)*	^{14}C-urea (count min^{-1} g^{-1} hr^{-1})	Urea synthesis† (μmole g^{-1} hr^{-1})
Neoceratodus X	None	0.01‡	382	3.8×10^{-3}
Neoceratodus X	Slices boiled	.001	34	
Neoceratodus X	L-Citrulline, $10^{-2}M$	.01	352	
Neoceratodus X	L-Arginine, $10^{-2}M$	.004	120	
Neoceratodus Y	None	.02	576	5.8×10^{-3}
Protopterus (4)§				6.9×10^{-1}

* [Count min^{-1} in urea/count min^{-1} in incubation medium] $\times$ 100. † Calculated by dividing (count min^{-1} g^{-1} hr^{-1}) by specific activity of ^{14}C-bicarbonate (10^5 count min^{-1} μmole^{-1}) in medium. ‡ Average of duplicate assays. § Six fish, average.